

Impact of substrate heterogeneity on anaerobic co-digestion process: a review

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Abstract

Anaerobic co-digestion is one potential strategy for maximizing the infrastructure's capacity for treatment while improving biogas output. It involves the addition of two or more substrates being digested simultaneously in the process. Anaerobic co-digestion's primary goal is to increase biogas, mostly bio-methane for domestic heating activities and electricity. By increasing bio-methane yields, anaerobic co-digestion becomes an effective and proficient method for reducing the limitations of mono-digestion and enhancing the commercial efficiency of existing anaerobic co-digestion amenities. By co-treating two or more waste streams, improved biogas generation can be accomplished through anaerobic co-digestion.

Keywords

Anaerobic Co-digestion, Substrate, Co-treatment, inhibitions, Biogas.

Introduction

As an established technology, anaerobic co-digestion (AnCo-D), is widely utilized to handle different organic waste streams, such as animal manures (Vögeli *et al.*, 2018), sewage sludge (Labatut & Pronto, 2018), food industry waste (Liebetrau *et al.*, 2019), energy crops like maize (Zhou *et al.*, 2015), and other agricultural residues. There are substantial financial and environmental advantages to capturing and burning the biogas produced by AnCo-D to replace fossil fuel energy use (Batstone & Jensen, 2011). However, some waste streams' weak degrading characteristics may pose difficulties for large-scale AnCo-D. Anaerobic Co-digestion is a method to take use of underutilized infrastructure's treatment capacity and increase biogas production (Hagos *et al.*, 2017; Zhang

et al., 2017; Keucken *et al.*, 2018). it is a procedure that improves biogas production by mixing different materials with waster water in a digester (Ahmed *et al.*, 2021). By combining the treatment of two or more waste streams that have complementary characteristics, usually by speeding up the loading rate within the existing AnCo-D systems, improved biogas production is made possible by AnCo-D (Budyh-Gorzna *et al.*, 2016). Possible substrate mixture synergism, diluted mixture toxicity, and increased digestate quality are some additional significant advantages of AnCo-D (Mata-Alvarez *et al.*, 2014, Shah *et al.*, 2015). For optimal biogas performance and process risk management, it is crucial to strategically construct combinations of AnCo-D waste composition complexity.

In anaerobic co-digestion the major objective is to produce more biogas, primarily bio-methane, for use as heat and electricity. A variety of feed stocks can be co-digested at an appropriate blend ratio to maintain the ideal conditions required for metabolic activity. To ensure the viability and sustainability of industrial anaerobic co-digestion, plants must combine process variables and substrates in the most effective manner possible. Several authors have used co-digestion to maximize the biogas production efficiency of substrates (Haider *et al.*, 2015, Owamah & Izinyon, 2015b). Anaerobic co-digestion process has reportedly improved due to improved carbon to nitrogen balance, good synergism for supporting mi-

crobial growth, increased production of biogas and co-digestion of various materials (Haider *et al.*, 2015). Processing them with more biodegradable wastes such as food remains or waste activated sludge (WAS) is a practical solution to increase the digesters' viability and to improve its biogas production.

These co-substrates ought to be easily and widely accessible close to the biogas plant (El-Mashad & Zhang, 2010). The dilution of hazardous compounds, nutrient balance, increased organic loading rate (OLR), and synergistic effect on microorganisms are just a few advantages of AnCo-D systems (Bayr *et al.*, 2014, Rabii *et al.*, 2019).

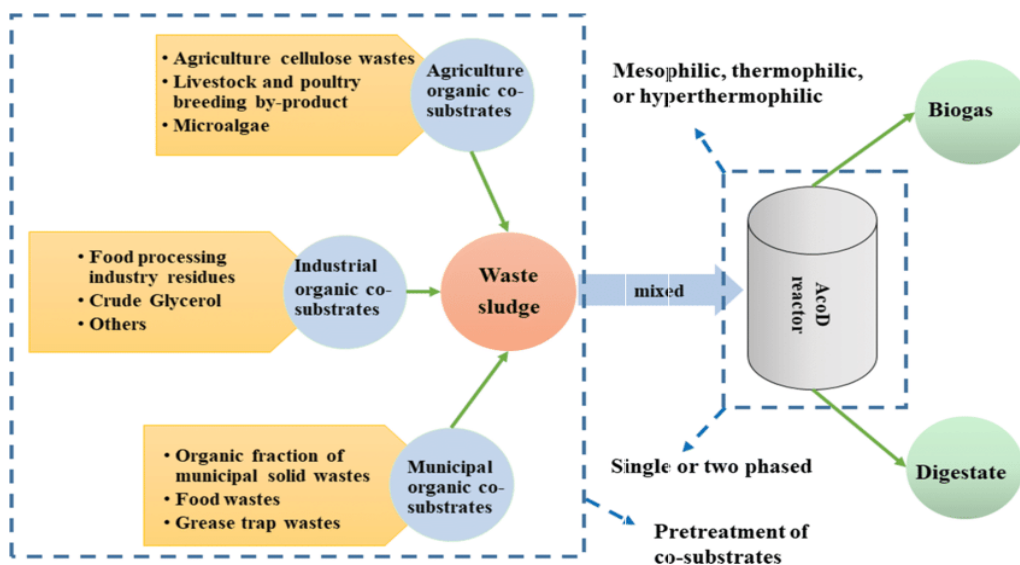


Figure 1

Shows sources of main organic materials in anaerobic co-digestion of waste activated sludge (Culled from Yang *et al.*, 2019).

Factors that impact on the anaerobic co-digestion process

The level of functional redundancy within the community is supported by a number of factors, including a decrease in pH, and increase in medium temperature, the deposition of microbial metabolites, the gradual depletion of available nutrients from the substrates, and the replacement by organisms that frequently use some of their byproducts.

Microbial Consortium

Creating biogas from organic substrates (biomass material) is intricate and reliant on microbes. The organisms that catalyse the conversion of organic substrates into biogas and other inorganic components engage in a variety of interconnected microbial activities that involve bacteria, archaea, and fungi

among other microbes. Thanks to the development of high throughput sequencing, the various microbial groups involved in the production of biogas have recently been identified (De Vrieze *et al.*, 2015, Sun *et al.*, 2015). The members of the microbial community in the system that are the most stable are fungi; despite being the group that is least prevalent in the microbial community, according to a recent study. They can ferment carbohydrates into acetate, carbon dioxide, formate, ethanol, hydrogen, and lactate because of their facultatively anaerobic nature. They are members of the phyla *Neocallimastigomycota*, *Mucoromycotina*, *Pucciniomycotina*, *Agaricomycotina*, *Saccharomycotina*, and *Peziizomycotina*. According to research, fungi and methanogens work best together to break down cellulose (Gruninger *et al.*, 2014, Kazda *et al.*, 2014). Meanwhile during the biogas production

process, the chemical conditions of the system change, which causes bacteria and archaea to become slightly unstable and fluctuate (Alvarado *et al.*, 2015, De vrieze *et al.*, 2012). Bacteria play a major role in the production of biogas because they produce substances (acetic acids) as a byproduct of their activities, which serve as a substrate for methanogens. These bacteria also include facultative anaerobes (*Clostridium*, *Paenibacillus*, *Ruminococcus*, *Streptococci*, and *Thermoanaerobacteriaceae*), sulfate-reducing bacteria (*Desulfovibrio*), and acidogenic bacteria (*Acidaminococcus* and *Aminobacterium*). Other bacterial groups that are known to participate in the process include *Proteobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Actinobacteria*, *Acidobacteria*, *Spirochaetes*, *Plantomycetes*, *Fibrobacteres*, *Tenericutes*, and *Cloacimonetes*. Few of these bacteria participate in the hydrolysis of organic molecules, although *Acidaminococcus* and *Desulfovibrio* are responsible for generating acetic acids from the substrates (Azman *et al.*, 2015, Li *et al.*, 2015, Rui *et al.*, 2015). The other syntrophic acetogens that break down and oxidize alcohols and organic acids into acetate, hydrogen, and carbon dioxide are called *Syntrophorhabdus*, *Syntrophus*, *Syntrophobacter*, and *Pelobacter*. They are widely distributed within the biogas digester. These cellulolytic microbes have the ability to convert cellulose into simple sugars (Worm *et al.*, 2014, Koeck *et al.*, 2014). The type of material used and the temperature of the biogas digester will determine which type of microbes predominate in the system. When temperatures are higher, *thermomagae* predominate. The most prevalent bacteria in the digester that contains animal waste and/or organic manure are called firmicutes. Municipal wastewater has higher levels of *Chloroflexi* at the substrate level than either organic manure or animal dung (Lebuhn *et al.*, 2014, Sundberg *et al.*, 2013, St-Pierre & Wright, 2014).

Preference of substrate

The type of substrate is more important than the pH, operating temperature, organic loading rate, hydraulic time, and digester design because microorganism acti-

vities directly depend on the nutritional composition of the substrate. The compositions of substrates can vary, which can cause issues in different systems. For example, substrates with high protein and fat content have a high energy content and consequently a high potential for methane production. These substrates always carry the risk of causing foaming or the production of inhibitory compounds, which can cause process disruption. While the risk of process disruption may be lower for some materials, such as lignocellulosic ones, their degradation process is unfeasible due to its extended duration. Plant-based foods include fruits, grains, vegetables, and root crops; these foods are often high in different types of polysaccharides. Polysaccharides are chains of sugar molecules that can branch (hemicellulose, pectin, and glycogen) or be linear (cellulose and starch). Plant cell walls contain lignocellulose, which is made up of hemicellulose, cellulose, and lignin (Rabii *et al.*, 2019). Simple polysaccharides like starch and glycogen can be swiftly broken down into glucose units by microorganisms. While lignin is mixed with hemicellulose and cellulose to form lignocellulose, the structure becomes relatively resistant to microbial degradation, just like in plants (Haider *et al.*, 2015, Owamah & Izinyon, 2015b). The most common renewable biomass sources are straw (wheat, rice, corn, and barley) and sugarcane bagasse, which have the potential to significantly boost biogas production worldwide. Dairy, animal manure, aquaculture sludge, slaughterhouse waste, and other animal-related wastes are rich sources of protein that can be used to produce biogas. The way in which proteins are broken down is influenced by the makeup and solubility of these substances (De Vrieze *et al.*, 2012). The theoretical estimation of the potential methane yield and the percentage of biogas that can be produced from nutrients is displayed in Table 1. Lipid-rich substrates, such as fats, have a higher potential for methane yield, as Table 1 illustrates. Long-chain fatty acids are released during its breakdown, though, and these may be harmful to microorganisms and cause pH levels to drop.

Table 1. Estimation of the maximum theoretical methane yield and biogas percentage composition

Nutrient	Methane Output (m ³ /kg VS)	CH ₄ (%)	CO ₂ (%)	Reference
Carbohydrate	0.42	51	50.5	Schnürer, 2016
Proteins	0.50	51	50.5	Schnürer, 2016
Lipid	1.01	71	30.5	McGenity et al., 2016

This might be lessened by using a start-up strategy that encourages the development of a specific subset of microorganisms that are resistant to toxicants (Rasit *et al.*, 2015, Mata-Alvarez *et al.*, 2014, Chen *et al.*, 2014). High protein substrates also have a high potential for methane yield. Such substrates break down and release ammonium (NH_4^+), which could raise the anaerobic digestion process's alkalinity. As a result, the digestate's value as a fertilizer will increase, and the methanogens' activities will be inhibited. More so, agricultural, municipal, and industrial organic wastes can be broadly categorized into three groups as substrates for the production of biogas.

Agricultural wastes

Agriculture-derived organic material is frequently used as a co-substratum in anaerobic digestion, including cellulose crops, energy plants, and livestock manure. On the other hand, agricultural cellulose residues—which include ground up grass, crop straw, coffee grounds, and other materials—are produced in huge quantities all over the world. Agricultural cellulose residues are primarily composed of three substances: cellulose, hemicellulose, and lignin. The unbalanced C/N ratio that arises from mono-digestion of waste activated sludge (WAS) can be corrected by co-digestion of cellulose, which has a high proportion of carbon by weight, and WAS, which has a high proportion of nitrogen by weight (Hidaka *et al.*, 2016,

2013). The amount of crude protein, carbohydrates, and macromolecules (like lignocellulose) in livestock manure varies greatly depending on the animal species (Zhang *et al.*, 2014, Borowski *et al.*, 2014).

Manure from pigs and chickens is high in protein. Contrarily, there is a lot of lignocellulose in cow manure. Multiple peaks rather than a single peak of methane production were produced by the anaerobic co-digestion of WAS with livestock and poultry breeding by-products (Zhang *et al.*, 2014, Kafle & Kim, 2013). Rich in rumen microbes, manure—typically the dung of sheep and cows—speeds up and improves the effectiveness of the anaerobic co-digestion process. Nonetheless, anaerobic digestion may benefit or suffer from the comparatively high nitrogen content of animal and poultry manure. However, methanogens were severely inhibited by high ammonia, which resulted in the build-up of volatile fatty acids (VFA) (Kafle & Kim, 2013). Anaerobic digestion technology transforms animal waste in a way that produces sustainable biogas energy and effective waste management practices. It has been determined that manure from cattle, pigs, sheep, goats, and chickens is a suitable substrate for the production of biogas. This is due to its high nutrient content, high organic matter content, and high buffering power. The physiochemical properties of various animal manures are shown in Table 2, along with the equivalent yield of methane.

Table 2. The methane yield and physiochemical properties of various animal manures

Animal Manure	pH	TS (%)	VS (%)	C\N Ratio	CH ₄ Output (mL/gVS)	Reference
Ruminant	7.1– 8.6	14.5- 22.7	11.9- 72.0	14.59–18.9	157.0–395.0	Li et al., 2020, Achinas et al., 2019, Shen et al., 2019
Pig	6.4- 7.5	8.2- 36.7	6.2- 82.8	5.7–13.5	204–438.4	Wang et al., 2020, Duan et al., 2019
Chicken	6.9- 7.4	20.0- 92.6	18.3- 84.1	7.5–9.75	160.0–396.0	Scarlat et al., 2018, Cheong et al., 2019
Sheep	7.16- 8.1	22.3- 40.0	18.7- 72.7	11.3–14.7	207.0–357.0	Achinas et al., 2018
Goat	7.9	33.7- 55.5	27.7- 89.4	18.0	402–500	Imeni et al., 2019
Donkey	6.8	19.8	14.4	-	380	Mukumba et al., 2016

Lignocellulosic Biomass

The annual production of lignocellulosic biomass is estimated to be 200 billion tons, making it a plentiful resource for the creation of sustainable energy. Anaerobic digestion, which has been demonstrated to

be less energy-intensive, can be used to convert a variety of lignocellulosic biomass into biogas (Ma *et al.*, 2019). The three primary components of lignocellulosic biomass are cellulose, hemicellulose, and lignin, each of which has a major effect on the

material's ability to decompose. All cell walls of plants contain cellulose, which is the most prevalent and important component. Cellulose typically makes up 35 to 50% of the total composition. About 20–35% of the total composition of lignocellulosic biomass is made up of hemicellulose being the second-most prominent fraction. Many polysaccharides, including xylan, glucomannan, glucuronoxylan, xyloglucan, and arabinoxylan, can be found in plant tissue, depending on the species (Koupaie *et al.*, 2019). Hemicellulose breakdown is mostly caused by an essential enzyme called xylanases, which cleaves the 1, 4 backbone of the xylan polymers. This significant enzyme can take on various forms, depending on the hydrolytic activi-

ty, action mechanisms, and substrate specificity. Hemicellulose and cellulose are enclosed by the third component, lignin, to form a hydrophobic, three-dimensional structure known as "Lignin-Carbohydrate Complexes" (LCC). According to Ma *et al.*, (2019), lignin's recalcitrant nature hinders its total breakdown under anaerobic conditions. However, a number of pretreatment techniques are usually employed to break the linkage between lignin and polysaccharides and increase the accessibility of hemicellulose and cellulose to microorganisms and hydrolytic enzymes. Some lignocellulosic biomass is shown in Table 3, along with the corresponding compositions of hemicellulose, cellulose, and lignin.

Table 3. Some lignocellulosic biomass's composition for anaerobic digestion

Biomass Type	Dry Weight %			Reference
	Cellulose	Hemicellulose	Lignin	
Aesculapius	38.0-45.0	12.0-13.0	25.0–37.0	Karthikeyan & Visvanathan, 2013
Switch grass	43.1	31.7	11.3	Li et al., 2013
Nut shell	25.0-30.0	25.0-30.0	30.0–40.0	Muktham et al., 2016
Grasses	25.0-40.0	35.0-50.0	10.0–30.0	Muktham et al., 2016
Corn stover	33.7	19.1	15.2	Liew et al., 2012
Bagasse	38.2	27.1	20.2	Karthikeyan & Visvanathan, 2013
Rice straw	37.8	29.6	14.8	Mustafa et al., 2016
Cotton stalk	50.4	15.6	16.3	Zhang et al., 2018
Wheat straw	48.6	29.4	7.3	Song & Zhang, 2015
Corn cob	45.0	35.0	1.05	Muktham et al., 2016
Rice husk	41.4	18.0	20.4	Li et al., 2013
Pineapple leaves	30.0	37.0	22.0	Mansora et al., 2019
Pineapple stem	37.0	34.0	20.0	Mansora et al., 2019
Pineapple root	42.0	32.0	19.0	Mansora et al., 2019

Table 3 shows the composition of all lignocellulosic biomass, such as corncobs, wheat straw, and cotton stalks. Except for eucalyptus and nutshell, lignin comprises the least amount of biomass. Since Aesculapius is a woody biomass, it has a high lignin content, which contributes to its high thermal stability. Table 3 makes it clear that not all of the biomass had a 100% composition of cellulose, hemicellulose, and lignin. A non-food source, faster growth rate, and adaptability of micro-algae make them one of the most viable sources of bio-diesel to substitute fuel derived from petroleum (Mandal *et al.*, 2009). Because algae contain less lignin (0.2–2%) and more readily hydrolyzed sugars (10–30%) and proteins (40–70%) than cellulose wastes, they are more easily

broken down during anaerobic digestion. Furthermore, it has been found that micro-algae contain micronutrients that are beneficial for methanogenesis, such as Fe, Co, and Zn, in addition to nutrients like C, N, and P (Ajeej *et al.*, 2015). According to Olsson *et al.*, (2014), co-digesting microalgae and sewage sludge from municipal waste water treatment in a VS ratio of 37:63 promoted biogas production in a mesophilic environment and raised methane yield by 23% over that of sewage sludge alone. Noteworthy is the fact that the production of toxic conditions at higher temperatures (thermophilic conditions) is facilitated by the release of extremely high concentrations of ammonia by protein-rich algae biomass. Consequently, the produ-

ction of biogas under thermophilic conditions was comparatively lower than under mesophilic conditions. Another study employed microalgae growing in municipal wastewater as a co-substrate for mesophilic digestion of sewage sludge. The addition of micro-algae increased the digested sludge's dewaterability rather than its methane production. Conversely, the methane yield decreased to 168 ± 22 ml/gVS from 200 ± 25 ml/gVS. The heavy metal content of the microalgae increased and inhibited the methanogen, resulting in a lower methane yield when heavy metal-rich flue gas was utilized as a source of CO₂ for growing microalgae (Olsson *et al.*, 2018).

Municipal food wastes

Anaerobic digestion of food waste alone has limitations on optimal degradation, digester stability and biogas yield (Shrestha *et al.*, 2023). Although more than one third of bio-degradable municipal solid waste (MSW) is dominated by food waste, food waste (FW) lacks in trace elements like Co, Fe, Ni, Zn for survival of methanogens in the anaerobic process (Mirmohamadsadeghi *et al.*, 2019, Braguglia *et al.*, 2018). FW alone adds process instability and process deterioration leading to lower biogas yield and even digester failure (Dhungana and Lohani, 2020). The co-digestion of FW with pretreated feed stock has shown beneficial impact on biogas generation. Co-digestion of FW with sludge from wastewater plant, green biomass waste and livestock manure has shown significant enhancement of up to 25-40% in biogas production (Mehariya *et al.*, 2018, Dhungana *et al.*, 2021). AnCo-D of FW could balance system stability by offering toxicity dilution, synergism and a robust microbiome (Ren *et al.*, 2019). According to Lee (2012), there were no beneficial effects on methane yield or the rate at which volatile solids were removed when WAS was co-digested with food and livestock wastewater. While the low-solids co-digestion system (total solid = 4.8%) did not show any synergistic phenomena, the highest synergistic effect was noted in the high-solids co-digestion of low-organic WAS and food waste (FW) (FW 50 vol%, TS 14%, pH 7.5-8.5). The co-digestion of sludge with different fruit wastes (peach, banana, and apple waste) was investigated in a semi-continuous reactor at 37°C, which is a mesophilic environment. When the type of co-substrate was changed, the quantity of VFAs did not change noticeably while the OLR stayed the same (Fonoll *et al.*, 2015). Due to varying biodegradability rates, the specific methane production (SMP) with

different fruit waste as co-substrates was 230–270 ml/g-VS, which was 110–180% that of mono-digestion of WAS. In recent years, lipid-rich wastes have become more and more popular as co-substrates for WAS. Anaerobic co-digestion may enhance the particular microbial activity. The co-digestion system was enhanced by a 40% increase in EPS release during the co-digestion of FOG with WAS (Yang *et al.*, 2016a). Increased EPS may provide more surface area for microbe colonization to adsorb, which is beneficial for biomass degradation. The main cause of the inhibitory problems with lipids is long-chain fatty acids (LCFAs), which are poorly soluble (Silvestre *et al.*, 2014).

Catalysts

Catalysts are crucial to the conversion of biomass because they can either improve the conversion products or advance the conversion processes. Zeolites, for example, have shown a great deal of promise in the processing of biomass, especially in the conversion of lignocellulosic biomass into chemicals and fuels. They are essential in converting oxygenates into hydrocarbons because they catalyze reactions such as esterification, decarboxylation, acylation, and dehydration. Consequently, the use of zeolite catalysts in the processing of biomass presents a viable alternative technique for the production of chemicals and fuels for transportation. Catalysts are also required for the gasification products. They reduce tar content, improve gas quality, and increase conversion effectiveness. Dolomite, alkaline metal oxides, and oxides based on nickel are common gasification catalysts. The addition of flocculants, adsorbing materials, surfactants, metal elements, enzymes, and other substances affects the yield of biogas, the nature of the co-digestate, and the overall effect (Yang *et al.*, 2017, Wang *et al.*, 2017c). Non-ionic surfactants, such as alkyl poly-glycoside (APG), were found to have a positive impact on anaerobic co-digestion during the mesophilic anaerobic co-digestion of green waste, FW, and WAS. However, at 15 mg/g, an adverse effect was observed, resulting in a modification of the microbial community's composition within the reactor (Sun *et al.*, 2019). Furthermore, Yang *et al.*, 2010 confirmed that the amount of dissolved organic matter and EPS in the digestion system are significantly affected by the addition of 0.06 g/g of mixed enzymes (protease/a-amylase, 1:3 w/w) to dry sludge.

Operating temperature

It has been possible to employ the co-digestion process in mesophilic, thermophilic, and even hyperthermophilic environments (Wang *et al.*, 2014a, 2014b). The presence or possible formation of inhibitory compounds, feeding strategy, substrate, and other operating factors should all be taken into account when determining the optimal operating temperature. Mesophilic (37–40°C) or thermophilic (50–55°C) temperatures are commonly used for digestion in industrial biogas processes. It has also been shown that temperatures between mesophilic and thermophilic (41–45°C) and psychrophilic (25°C) are feasible. In comparison to mesophilic conditions, thermophilic conditions typically produce significantly more biogas and have a higher endurable OLR value (Li *et al.*, 2017, Gou *et al.*, 2014). Increased thermophilic bacterial growth rates and quicker biochemical reaction rates may be to blame for the rise in biogas production and OLR. The solubilization of substrates into products is enhanced at higher temperatures, which also makes co-substrates easily decomposable, enhancing the mixture's biodegradability. It therefore follows that different temperature conditions are required to match with different substrates, due to characteristics of each substrate (Chow *et al.*, 2020). The rate of hydrolysis can increase under thermophilic conditions. High temperatures also reduce the amount of pathogens in effluents (Kim *et al.*, 2011). Nevertheless, thermophilic anaerobic co-digestion has certain disadvantages as well, such as low stability, sensitivity to inhibitors, high energy requirements, high volatile organic compound residue in the wastewater, and poor dewaterability. Bacteria that produce methane may be inhibited by increased concentrations of nutrients produced under thermophilic conditions and subsequently higher concentrations of VFA or NH₃ (Montanes *et al.*, 2015). The microbial population can fluctuate over time and go through periods of instability as a result of temperature changes. Therefore, it is preferable to gradually raise or lower the temperature (by no more than one degree every day) to allow the population to adjust. Kabouris *et al.*, 2009 showed that a mesophilic condition could achieve a greater improvement at 198% of methane yield compared to thermophilic at 169%. Temperature variations must be closely watched to prevent process collapse, both when raising and lowering the working temperature. In the event of disturbance during step-wise temperature changes, a temporary decrease in

feed rate and an extension of the retention time may be necessary.

Pretreatment techniques

Pretreatment has unique impacts on various co-digestion biomass due to the varying substrate composition (De la Rubia *et al.*, 2018, Naran *et al.*, 2016). One effective technique for handling solid waste is mechanical biological treatment (MBT). According to Velis *et al.*, (2009), it is composed of two processing units: one for mechanical processing, such as air classification and crushing, and another for biological conversion, such as anaerobic digestion or composting. The advantage of MBT is that the pretreated material's quality satisfies processing requirements (suitable physical composition, acceptable levels of heavy metals, and other contaminants), and the substrate's post-MBT size and biomass reduction make it better suited for co-digestion. In a study, the methane content and biogas output were, respectively, 290 mL/gVS and 35% in the control (100% primary sludge), 130 mL/gVS and 43% (25% MBT products), and 240 mL/gVS and 47% (12.5% MBT products). The MBT products increased the methane content of the biogas, even though a synergistic effect on biogas generation was not observed during co-digestion (Pahl *et al.*, 2008). Micro-algae were thermally pretreated for 10 hours at 75 degrees Celsius, which sped up the release of inhibitory compounds and raised the risk of phytotoxicity. However, co-digestion reduced these negative effects because WAS diluted the mixture. In the interim, co-digestion was employed to homogenize the co-digestate and stabilize the fermentation (Sole-Bundo *et al.*, 2017).

Co-digestion of various lignocellulosic biomass material

In a study, wheat straw was pretreated with four different concentrations of H₂O₂ (1%, 2%, 3%, and 4%), and it was used as a mono- and co-substrate in various ratios with dairy cattle manure before being digested (Song & Zhang, 2015). Methane yields for the 1%, 2%, 3%, and 4% pretreatment of H₂O₂-treated wheat straw in mono-digestion were 94.8, 108.5, 128.4, and 118.7 mL/gVS, respectively, while the untreated wheat straw yielded 84.3 mL/gVS. The methane yield considerably rose when cow dung and wheat straw—both of which had received H₂O₂ treatment—were digested simultaneously. The highest methane yield was produced by co-digesting untreated

wheat straw and cattle manure at a mixing ratio of 30:70, resulting in 257.6 mL/gVS of methane. In contrast, methane from co-digesting H₂O₂-treated wheat straw and cattle manure at a mixing ratio of 40:60 produced the highest methane yield of 320.8 mL/gVS. Almomani and Bhosale (2020) conducted a study that proposed the addition of cow dung to

certain agricultural solid wastes, including wheat straw, grass, and clover, in order to enhance their biogas yield. The maximum cumulative methane production (CMP) of 297.99 NL/kg VS was obtained by mixing agricultural solid wastes and cow dung in a 60:40 ratio, as specified in Table 4.

Table 4. A summary on some studies of co-digestion of lignocellulose and animal manure

Substrate type	Mixing ratio	pH	Pretreatment	CH ₄ Output (mL/gVS)	Reference
Wheat straw + cattle manure	41:60	6.5-7.0	3%w/w H ₂ O ₂	320.8	Sun et al., 2015
Wheat straw +cattle manure	30:71	6.8-7.1	None	254.6	Sun et al., 2015
ASW + Cow dung	62:40	8.1	None	297.7	Sundberg et al., 2013
ASW +Cow dung	60:43	8.1	NaHCO ₃ /g	386.3	Sundberg et al., 2013
Corn Stover + Chicken manure	3:1.5	6.9-8.2	Wet- AD	218.8	Tien & Sim, 2012
Corn Stover + chicken manure	1:1	8.0-9.3	SS-AD	147.8	Velis et al., 2009
Goat manure + Corn stalk	70:30	6.5-7.5	None	16.0	Velis et al., 2009
Goat manure + rice straw	50:50	6.5-6.8	None	15.7	Velis et al., 2009
Sugarcane bagasse+ cow dung	1:2	6.8	2% w/w NaOH	386	Vogeli et al., 2014
Sugarcane bagasse +cow dung	1:2	6.8	None	322	Vogeli et al., 2014

In another study, the co-digestion of rice straw and cow manure was investigated in relation to the organic loading rate using a continuous feeding mechanism. A batch test analysis was performed before the continuous experiment to establish the optimal mixing ratio of 1:1 for the volatile solids. With an organic loading rate of 6 g/L d, an efficient and stable co-digestion was accomplished with an average biogas production and daily volumetric biogas production rate of 383.5 L/kg VS and 2.3 m³/day, respectively. The accumulation of VFA brought on by an additional rise in organic loading significantly hampered the co-digestion process (Li *et al.*, 2013). Corn stover, the residue usually left over after harvesting maize, was co-digested with chicken manure in three different anaerobic digestion conditions: hemi-solid state (HSS-AD), wet (W-AD), and solid state (SS-AD) (Li *et al.*, 2013). Corn stover is a potential biogas substrate. To maximize methane production and achieve process stability, the study set out to determine the optimal mixing ratio for each of the three anaerobic digestion conditions (Li *et al.*, 2013). For corn stover and chicken manure, an ideal methane yield of 218.8 mL/gVS and 208.2 mL/gVS, respectively, occurred at a substrate-mixing ratio of 3:1 during wet and hemi-solid state anaerobic digestion conditions. With a 1:1 mixing ratio, the ma-

ximum volumetric methane productivity was 14.2 L methane/reactor volumes. Furthermore, combining the substrates in the ratios of 3:1 and 1:1 under solid-state conditions produced a synergistic effect. The highest biogas yields were obtained with mixing ratios of 30:70, 70:30, and 50:50 for goat manure/wheat straw, goat manure/corn stalk, and goat manure/rice stalk, respectively. The combined biogas yield of 12.8 L/kg VS from the co-digestion of goat manure and wheat straw at a mixing ratio of 30:70 was 23.0% and 62.1% higher than their single-mode. However, compared to separate digestion of rice straw and goat manure, co-digestion of the two materials at a 50:50 mixing ratio resulted in a total biogas yield of 15.7 L/kg VS, which is higher by 111.28% and 51.31%. While the yield from co-digesting corn stalk and goat manure is 54.44% and 83.02% higher than the yield from the two materials alone, the combined biogas yield of 16.0 L/kg VS is produced. The carbon to nitrogen imbalance caused by single substrates could be overcome by co-digestion, which greatly increased the amount of biogas produced. The use of additives also comes in handy. Biochar enhances the performance of anaerobic co-digestion and produces more biogas in general (Liu *et al.*, 2021, Xiao *et al.*, 2021, 2020). Biochar enhances the buffering capacity, alleviates ammonia acid inhibition improving micro-

bial enrichment (Qui *et al.*, 2019). Li *et al.*, 2022 hypothesized that biochar residues could be recycled into new biochar and added into the anaerobic co-digestion of sewage sludge and food wastes. The result showed that residue biochar produced the highest daily methane amount of 432.2ml g/VS versus 377.7-386.3ml for coconut and corn biochars. It turned out that residue biochar neutralized fatty acids thereby hindering acidification. More so, higher abundance of sludge *Clostridia*, *Methanobacterium* and *Methanobrevibacter* accelerated methanation. Microalgae can be used as a co-substrate in the anaerobic digestion of waste activated sludge (WAS). When sewage sludge and microalgae from municipal waste water treatment were co-digested in a VS ratio of 37:63, methane yield rose to 408 ± 16 ml, or 23% more than when sewage sludge was used alone (Ajeej *et al.*, 2015, Olsson *et al.*, 2014). This promoted biogas production in a mesophilic environment. The release of extremely high concentrations of ammonia by protein-rich algae biomass is an important point to note because it facilitates the creation of a toxic environment at higher temperatures (thermophilic conditions). Consequently, the production of biogas under thermophilic conditions was comparatively lower than under mesophilic conditions. Another study employed microalgae growing in municipal wastewater as a co-substrate for mesophilic digestion of sewage sludge. Micro-algae increased the dewaterability of digested sludge rather than increasing methane production. Conversely, the methane yield decreased to 168 ± 22 ml/g VS from 200 ± 25 ml/g VS. Flue gas rich in heavy metals was used to grow micro-algae; however, the high heavy metal content of the micro-algae inhibited the methanogen, resulting in a lower methane yield (Olsson *et al.*, 2018). In a study by Zongo *et al.*, 2023, algal-bacterial biomass are cultured on high-strength waste water and then co-digested with sugar bagasse. Although methane content of biogas were similar in all digestion sets (61-67%), co-digestion sets with algae and bagasse produced higher methane yields (145 and 101ml CH₄/g VS) than algae alone (61 and 82ml CH₄/g VS) or bagasse alone (74ml CH₄/g VS). A significant correlation ($r^2 = 0.88$, $p = 0.012$) was observed between algal-bacterial biomass content of the substrate and total gas production and therefore methane yield. In terms of methane yield and VS removal rate, co-digesting WAS with food wastewater and livestock wastewater did not have a synergistic effect (Lee, 2012). While the low-solids co-digestion system (total

solid=4.8%) did not show any synergistic phenomena, the highest synergistic effect was noted in the high-solids co-digestion of low-organic WAS and food waste (FW) (FW 50 vol%, TS 14%, pH 7.5-8.5). Peach, banana, and apple waste were among the fruit wastes that were co-digested with sludge in a semi-continuous reactor at 37° C, which was mesophilic. When the organic loading rate was held constant and the co-substrate was changed, there was no appreciable change in the quantity of VFAs (Fonoll *et al.*, 2015). Due to varying biodegradability rates, the specific methane production (SMP) with different fruit waste as co-substrates was 230–270 ml/g-VS, which was 110–180% higher than that of mono-digestion of WAS. Investigations were conducted into the mesophilic anaerobic co-digestion of waste activated sludge and olive mill wastewater (OMW). The highly toxic phenols in OMW may inhibit the anaerobic processes that cause methanogenesis and biodegradation, but co-digestion of WAS produced 77 L/d of biogas, a 157% increase over mono-digestion of WAS. The estimated value of the increase was 326.1% (Athanasoulia *et al.*, 2012b, Boari *et al.*, 1993). Glycerine was added to the co-digestion of sewage sludge in order to increase the C/N ratio. This is beneficial for the synthesis of extracellular polymeric substances (EPS), but it decreased the dewaterability of the digestate (Nartker *et al.*, 2014, Silvestre *et al.*, 2015). Sugarcane bagasse is another agricultural waste that can be utilized as a substrate for co-digestion because of its energy potential. It is a waste product created when the crop of sugarcane is milled. A recent study examined the anaerobic co-digestion of cow dung and pretreated sugarcane bagasse. The bagasse was pretreated with a solution of NaOH and Ca(OH)₂ for one day prior to being combined 1:2 with cow dung. Table 4 shows that at 35 °C, untreated bagasse yielded approximately 322 mL/g VS of biogas, whereas bagasse treated with Ca(OH)₂ yielded a maximum of 386 mL/g VS. By mixing cow dung with pure bagasse and raising the temperature from 35 to 55 degrees Celsius, the biogas yield rose by 27 milliliters per gram of solid waste. This could be explained by the fact that pure sugarcane bagasse's 130:1 carbon/nitrogen ratio changed to 29:1 upon the addition of cow dung (Kaur *et al.*, 2020). When pretreated sugarcane bagasse and cow dung are co-digested together, the biogas yield is higher than when pure sugarcane bagasse is used because of the increased internal surface area and decreased degree of lignocellulose polymerization. This in turn causes

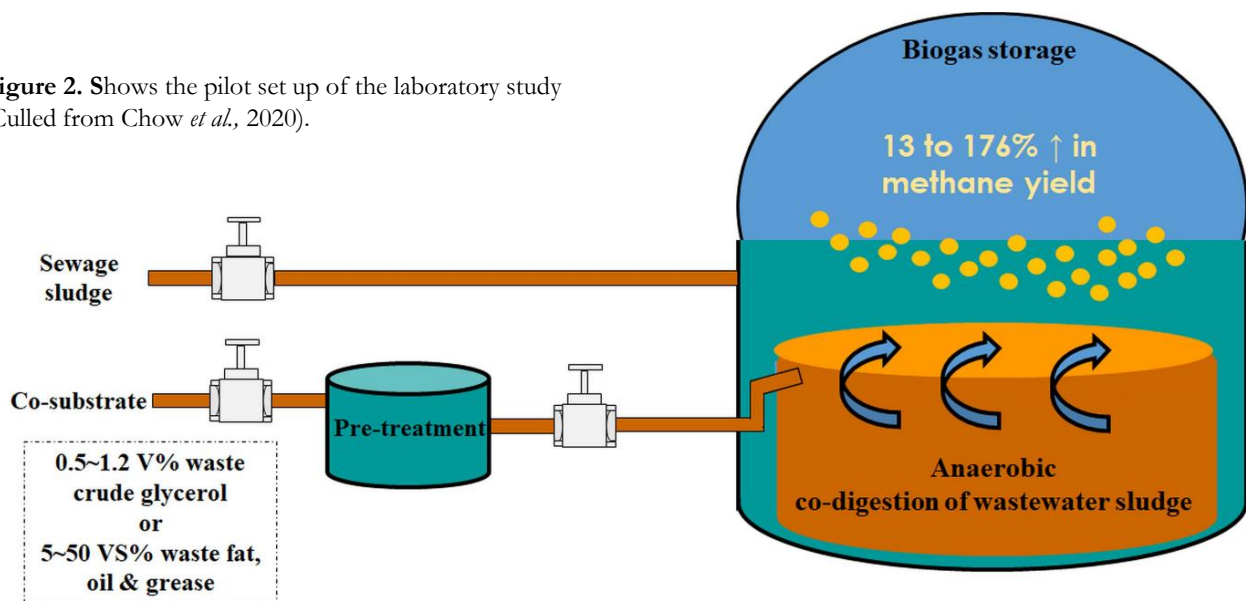
the bond between the lignin and carbohydrates to dissolve due to the alkaline pretreatment that was applied. An investigation was conducted to create an assay for the co-digestion of animal manure with silage made of grape byproduct, tomato pulp, and olive agro-food byproduct. A blend of 45% VS calf manure, 41% VS lamb manure, and 2% VS pig manure made up the animal manure. The study discovered that when animal manure and tomato pulp were co-digested, a higher methane yield of 404 mL/g VS was obtained, indicating a greater synergistic effect than with grape byproduct and olive agro-food byproduct. The highest methane yield was found when there was a greater ratio of animal manure to agro-food byproducts. An increase in the chemical oxygen demand of the assay could account for this. Alkaline parameters and ammonia nitrogen were also found to be correlated at higher ratios of animal waste (Parralejo *et al.*, 2019). A mathematical model was developed to assess the performance potential of a biogas digester that is fed specific kinds of substrate (Mukumba *et al.*, 2019). It has been reported to have a 75% methane percentage composition with an equal mixture of horse, goat, donkey, and cow dung. The reviewed studies show that co-digestion of agricultural wastes and animal manure enhances the production of biogas and stabilizes the anaerobic digestion process. This is because nutrients are more uniformly distributed as a result of co-digestion, particularly between carbon and nitrogen. e to the quick breakdown of kitchen waste (KW) and the buffering properties of poultry manure (PM), anaerobic co-digestion of KW and PM produced cumulative biogas (CBG) in a different study (Rahman *et al.*, 2021). Digester 1 (D1), loaded with only KW, produced 312 ± 9 ml of CBG during the 24-day experiment at room temperature of 28 °C, while Digester 2 (D2), loaded with equal amounts of KW and PM, produced 362 ± 13 ml of CBG. Digester 2 therefore produced 16% more biogas than Digester 1 (D1) at the same temperature of 28 °C and with the same amount of inoculum—50 g Cow Manure. This increase in gas output could be the result of the synergistic effect of co-digesting KW and PM in a single digester. This synergistic effect is produced by the easy biodegradability of KW, which is required for increased substrate availability to be converted into biogas, and the increase in alkalinity provided by PM, which is required to maintain the pH level near neutral, which is required for the survival of methanogenic bacteria (Chuenchart *et al.*, 2020).

The pH decreased more quickly in D1 due to the KW's rapid breakdown, which may have released more VFAs (Wang *et al.*, 2014); however, in D2, the acidity was somewhat counteracted by the ammonia released during PM decomposition, which maintained the pH higher than in D1. Due to its high VS content, the KW rapidly hydrolyzes during digestion, severely acidifying the digester, suppressing methanogen activity, and producing less gas (Ye *et al.*, 2013, Jiang *et al.*, 2012). On the other hand, alkaline materials like ammonia and ammonium ions are produced when proteins and urea break down in PM, giving the digester greater buffering capacity. The additional buffering capacity supplied by PM helps to increase the production of biogas and stabilizes the system by reducing the negative effects of VFAs on methanogens (Abouelenien *et al.*, 2009, Kafle & Kim, 2013). Furthermore, PM was added to KW to raise the C/N ratio of the mixture to 22.35, which creates an environment that is ideal for methanogenic bacteria. Poultry litter was added to vegetable processing waste to raise the C/N ratios in the 13–28 range—the optimal range for anaerobic digestion (Bres *et al.*, 2018, Li *et al.*, 2013). The amount of biogas produced from municipal sewage sludge increased 1.5 times when co-digested with 30% PM (Borowski & Weatherley, 2013). In another study, wheat straw by itself produced 389.7 ± 24.7 ml/g VS of biogas; however, when combined with PM, it increased to 317.5 ± 31.3 ml/g VS (Wang *et al.*, 2012). The study suggests that co-digestion of KW and PM in a mesophilic environment could be a viable approach to improve the methane composition of biogas produced while maintaining the stability, buffering capacity, and nutrient balance of the digester. The substrate's pH and C/N ratio improved when food waste and rice husk were co-digested. Compared to Digesters B-3, B-4, and B-5 with higher I/S ratios, Digesters B-1 and B-2 with lower ratios performed worse at startup. This could be because there are more active methanogens, which shortens the time it takes for methanogenic populations to generate biogas (Haider *et al.*, 2015). Combinations of substrate were made in the following order: a 1:1 ratio of banana peel to vegetable waste, a 1:1 ratio of pig dung to banana peel, a 1:1 ratio of vegetable waste to pig dung, and a 1:1:1 ratio of banana peel to vegetable waste and pig dung. Then, before each mixture was put into the digesters, distilled water was added in a 1:2 ratio. Samples from the digesters were collected every 24 hours for 30 days during the digestion

process. By measuring the displacement of paraffin oil in a gasometric chamber, the daily volume of biogas produced was calculated. The biogas yield was highly influenced by the substrate treatments and the digestion duration (days) ($p = 00.5$). The maximum biogas yield was reached after 25 days of digestion; the volume of the biogas ranged from 45.58 cm³ (pig dung) to 58.90 cm³ (vegetable waste, banana peel, and pig dung). The methane yield varied between the fourth and fifth weeks (30–35 days) of digestion. During this anaerobic digestion process, the digester's mean temperature varied between 28° C and 39° C relative to the ambient temperature (temperature prior to digestion). A research by Chow *et al.*, 2020, identified certain co-substrates for waste water sludge anaerobic co-digestion.

By adding 0.5-1.2V% waste crude glycerol and a percentage of waste fat, oil and grease, a gain ranging from 13 to 176% in the methane yield was accomplished compared to mono-digestion. Figure 2 shows the pilot set up of the laboratory study. The key benefits of co-digestion are the adjustments of C/N ratio to optimum level, supply of lacking nutrients or trace elements and synergistic effects which trigger higher methane yield (Liao *et al.*, 2014). Co-digestion boosts hydrolysis process kinetic (Ebner *et al.*, 2016, Zamanzadeh *et al.*, 2017). The success of the co-digestion process depends largely on selecting suitable co-substrates and coming up with appropriate mixing ratio. Inhibition substances or toxic compounds present in one substrate can be diluted by adding co-substrates (Zamanzadeh *et al.*, 2017).

Figure 2. Shows the pilot set up of the laboratory study (Culled from Chow *et al.*, 2020).



Relieving inhibitions in Anaerobic Co-Digestion systems

Inhibitions are common phenomena in anaerobic digestion of various substrates (Arelli *et al.*, 2018, Ren *et al.*, 2018). They could be triggered by volatile fatty acid (VFA) accumulation, long chain fatty acids (LCFA) accumulation and free ammonia accumulation which can inhibit the growth of microorganisms hence lowering biogas yield. AnCo-D can lessen inhibition by dilution, system pH regulation, and alkalinity enhancement. However, overloading of co-substrates can equally cause inhibition or system failure, which stops the production of biogas. It is necessary to control inhibition in AnCo-D in order to optimize biogas production while maintaining performance stability. The build-up of volatile fatty acids (VFA) due to high organic loading has been found to be a significant risk factor for AnCo-D (Wang *et al.*, 2013). High VFA concen-

trations have the potential to inhibit anaerobic microorganisms. The pH of the system is lowered by excessive VFA, and the toxicity of undissociated species can permeate cells and have an impact on the related microbes. Accordingly, VFA inhibition is correlated with the systems pH and alkalinity (Yang *et al.*, 2015). According to Rajagopal *et al.*, 2013, AnCo-D feed stocks with high protein, urea, and nucleic acid content produces free ammonia (NH₃). AnCo-D can reduce ammonia inhibition by diluting the solution, adding substrates, or lowering the pH by accumulating VFA and quickly biodegradable co-substrates (Mata-Alvarez *et al.*, 2014). The bacterial cell will have to give up protons to maintain an ideal pH if NH₃ penetrates the cell membrane, increasing the internal ammonia concentration and ultimately leading to the cell's death (Ho *et al.*, 2014). Due to their simpler membrane structure than gram-negative bacteria, gram-positive bacte-

ria are more susceptible to NH_3 and less resistant to ammonia inhibition/toxicity. It is interesting to observe that gram-positive bacteria belonging to the phylum *Actinobacteria* decreased following the addition of grease (Razaviarani & Buchanan, 2014). Ultimately, the mechanisms of inhibition caused by the build-up of VFA, free ammonia, and LCFA are different, and the biomass's adaptation can significantly affect the inhibitory concentrations. Ren *et al.*, 2019 observed that in order to ease VFA accumulation, temperature, substrate to inoculum ratio (SIR) and pH need to be controlled in different stages of the anaerobic co-digestion stages. This adjustment strategy relieved VFA inhibition during AnCo-D of food waste and cow manure.

Conclusions

This review introduced the effects of heterogeneity on co-digestion under anaerobic conditions with different factors influencing the rate of biogas production. The way that each type of substance is composed affects how the anaerobic co-digestion process works. Thus, proper regulation and mixing strategies are needed for different kinds of substrates. For the combination of three or more different wastes and the simultaneous bio-degradation of new co-contaminants, more heuristic research is needed. The reactor's nutrient load is increased by the co-digestion of waste activated sludge and other organic wastes; the additional phosphorus and nitrogen could make the co-digestion system unstable. Biochar and nanoparticles, which have superior material qualities, could be added to address this problem. The type of substrate that is digested, the operating pH, temperature, organic loading rate, hydraulic retention time, and digester design all affect the anaerobic co-digestion process. In order to optimize the growth and functionality of microorganisms and enhance the biogas production process, the substrate must contain essential organic and mineral nutrients. The concentrations and availability of these macromolecules, micro-elements, and vitamins vary in substrates. Rich substrates in lipids and proteins yield more methane than substrates high in carbohydrates. The high lipid content of substrates causes the anaerobic digestive system to fail by forming long-chain fatty acids. Moreover, carbohydrate-rich substrates have the ability to alter the C/N ratio, resulting in nutrient restriction and quick acidification. A range of substrates is recommended to achieve nutrient balance, process stability, and increased biogas yield. According to this review, the selection of substrates for anaerobic co-digestion should be based on their availability, nutrient composition, and availability of pretreatment treatment options in order to maximize the biogas yield. Future research ought to consider supplementing the organic wastes with a small amount of inorganic fertilizer to encourage microbial growth. For a given volume of digested organic waste, this could result in a higher biogas yield.

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