

Review

A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration

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Abstract: Recent studies have shown that anaerobic co-digestion (AnCoD) is superior to conventional anaerobic digestion (AD). The benefits of enhanced bioenergy production and solids reduction using co-substrates have attracted researchers to study the co-digestion technology and to better understand the effect of multi substrates on digester performance. This review will discuss the results of such studies with the main focus on: (1) generally the advantages of co-digestion over mono-digestion in terms of system stability, bioenergy, and solids reduction; (2) microbial consortia diversity and their synergistic impact on biogas improvement; (3) the effect of digester mode, i.e., multi-stage versus single stage digestion on AnCoD. It is essential to note that the studies reported improvement in the synergy and diverse microbial consortia when using co-digestion technologies, in addition to higher biomethane yield when using two-stage mode. A good example would be the co-digestion of biodiesel waste and glycerin with municipal waste sludge in a two-stage reactor resulting in 100% increase of biogas and 120% increase in the methane content of the produced biogas with microbial population dominated by *Methanosaeta* and *Methanomicrobium*.

Keywords: anaerobic co-digestion; microbial consortia; digester mode; biogas; biomethane

1. Introduction

Waste materials including biowaste are constantly being generated due to inevitable human activities. Different techniques have been employed to manage and reduce the growing amount of biowaste. Incineration and gasification are the two main waste to energy technologies that have been widely used throughout the world. However, such technologies result in secondary environmental impact. Landfilling can lead to soil and groundwater contamination, imposing further actions and cost to remediate the contamination. It also requires large areas of land [1]. If not well managed and maintained, incineration will cause air pollution and subsequent environmental and health impacts. A number of detrimental products, such as dust, dioxins, furans, heavy metals, SO_x, NO_x, HCl, and HF are generated during the incineration process. A flue gas cleaning system is necessary to meet the regulatory requirements, while the need for using chemicals increases the cost of the process [2]. The incineration of some sources of waste, including animal waste, with a high moisture content and quite low calorific value, requires a certain amount of supplementary fuel [3]. Furthermore, a net positive energy balance can be obtained from combustion only if the moisture content of the biomass is lower than 60%. In pyrolysis and gasification, the energetic efficiency reduces with higher moisture content and the presence of water in the produced bio-oil is disadvantageous. The utilization of these technologies demands an energy consuming pre-drying stage to reduce the moisture content of the

wet type of biowaste. In contrast, anaerobic digestion (AD) is a microbial process that converts biomass to energy in an aqueous environment. Biomass sources, even with less than 40% dry matter content, can be used in the AD process. Recent studies verify a higher potential for improving AD systems in comparison with the incineration technology [4,5].

Mitigation of climate change and fossil fuel consumption requires a shift to alternative, renewable energy sources [6,7]. As reported by International Energy Outlook, 2011 [8], total world energy consumption will increase from 5.32×10^{20} J in 2008 to an estimated number of 6.35×10^{20} J in 2020, and it is expected to rise to 8.12×10^{20} J in 2035, which is equivalent to a 53% increase in the span of 27 years. Energy obtained from biomass is regarded as an important future renewable source, as it is capable of providing a continuous power generation and it is also an essential part of the current CO₂-mitigation policy [4,7]. Production of biofuel from biomass has received increasing attention during recent years. Several treatment processes and technologies have been established to obtain sustainable and affordable biofuel, for example Syngas (SNG) is a synthetic gas produced by gasification of a carbon containing fuel that has some energy value. However, production of SNG is narrowly practiced due to the high cost of this process [9,10].

Anaerobic digestion (AD), which is widely used for the treatment of wet residual biomass, is considered to be one of the most favorable processes for biofuel production from biomass. AD technologies can be categorized into three main classes based on the total solids (TS) content of the substrate: wet AD with TS < 15%; dry AD with TS < 25%; and solid state AD operated with a TS content of up to 40%. Currently, wet and dry anaerobic digestion techniques are mostly used for the organic fraction of municipal solid waste [11]. During the AD process, the organic matter will be decomposed by a microbial consortium in an oxygen-free environment. The main outcome of the AD process is the production of methane (50–75%), carbon dioxide (19–34%), and a small portion of biohydrogen (<1%) [7,12]. Methane from AD can be utilized as an energy source alternative to fossil fuels; the energy capacity can vary, depending on the type of waste, from 20 to 300 kWh of net energy per ton of waste [7,13,14]. According to the ministry of agriculture of Ontario, 48 kWh from dairy manure and 335 kWh from corn silage was obtained in 2016. The AD biological process is based on the biochemical conversion of organic matter into methane, which occurs through the metabolic action of methanogenic *Bacteria* and *Archaea* [15]. The metabolic reactions during AD include four stages, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are conducted by various groups of microorganisms. Complex organic compounds are first hydrolyzed through enzymatic reactions to monomers, such as glucose, amino acids, and long chain fatty acids (LCFAs), and they are subsequently converted in the acidogenic pathway to volatile fatty acids, H₂, and acetic acid. During the metabolic pathway of β oxidation, LCFAs break down in multiple stages to H₂. The acetogenic bacteria convert volatile fatty acids to H₂, CO₂, and acetic acid in acetogenesis stage. In Homoacetogenesis, Hydrogen is used to reduce carbon dioxide to acetate. Finally, methanogens convert H₂, CO₂, and acetate to CH₄ and CO₂ [16,17]. These pathways are summarized and shown in Figure 1.

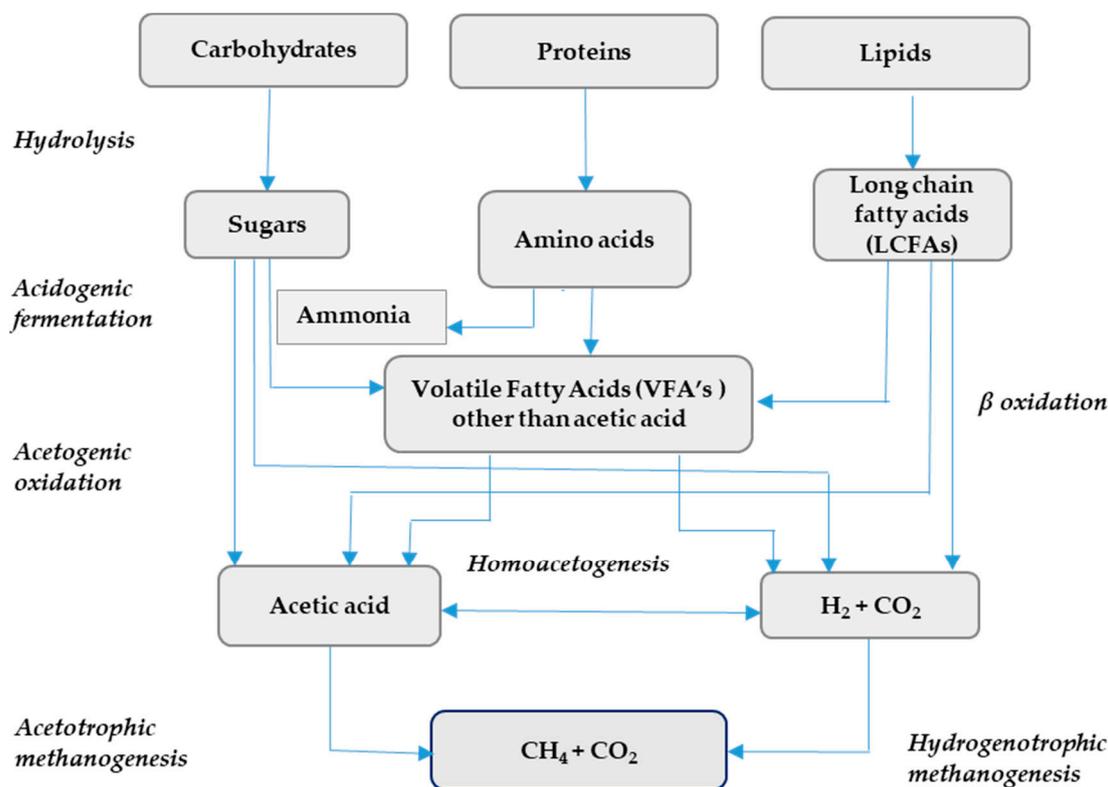


Figure 1. Pathways of anaerobic digestion for biomethane production.

Various sources of waste with high biological pollution loads, including organic fraction of municipal solid waste (OFMSW), agricultural and animal wastes, sewage sludge, and rural and slaughterhouse effluents, can be utilized as feedstock [18–20]. The capture of CO₂ and recovery of energy from biogas can contribute to greenhouse gas reductions by a considerable amount [7,20]. Moreover, digestate, the semi-solid residue of AD, contains demineralized nitrogen and phosphorus that can be utilized as organic fertilizer [21].

AD technologies have shown sufficient adaptability to different feedstocks [22]. Although AD is a commercial reality for a range of wastes, anaerobic digestion of single waste may be associated with certain drawbacks, such as unbalanced nutrients, rapid acidogenesis, poor buffering capacity, high ammonia nitrogen concentration, and inhibition of long chain fatty acids, which can inhibit methanogenesis and lead to severe instability and process disruption [23–25].

The growth rate of anaerobic microorganisms and subsequent biogas production depends highly on the composition of the organic matter in the feedstock. The constituents of the feedstock added to the digester are consumed selectively by a range of different microbial consortia. In addition, the existence of nitrogen in the feedstock is necessary for the synthesis of amino acids, proteins, and nucleic acids. It is also required for ammonia formation to neutralize volatile fatty acids (VFAs) produced during the fermentation process and to maintain neutral pH conditions for cell growth. However, an excess of nitrogen in the feedstocks can result in toxic effects to bacteria by extreme ammonia formation. Therefore, a suitable amount of nitrogen is required to provide sufficient nutrients while avoiding ammonia toxicity [26,27].

During anaerobic digestion, a series of complex biological degradation pathways are involved, which are influenced by numerous factors. Therefore, a profound understanding of the biochemical activities of anaerobic microorganisms in the AD system is required to support an effective control of the governing factors [28].

AD Process and Main Parameters

Overall, two groups of parameters can affect the anaerobic digestion performance, including environmental and operational factors. Environmental factors comprise temperature, pH, alkalinity, and waste characteristics, such as the amounts of volatile solids (VS), carbon to nitrogen (C/N) ratio, total solids (TS), nutrients, organic loading rate (OLR), ammonia, and VFAs. For example, VS contains both biodegradable volatile solids (BVS) and the refractory volatile solids (RVS) fractions. BVS fractions of substrates are helpful in better biodegradability of the waste, organic loading rate, C/N ratio, and biogas production. Waste materials containing high VS and low non-biodegradable material, or RVS, are the most suited to AD treatment. Only the biodegradable fraction of the VS has the potential for bioconversion. The refractory volatile solids in most feedstocks contain mostly lignin. Lignin is a complex organic material that is not easily degraded by anaerobic bacteria, and normally requires a long period of time for complete degradation [29,30].

The amount of carbon and nitrogen present in feedstock or the C/N ratio is a very important parameter for AD. A high C/N ratio leads to deficiency in the AD system, since it indicates rapid consumption of nitrogen by methanogens and leads to lower gas production. On the contrary, a lower C/N ratio results in accumulation of ammonia and exceeding pH values that are toxic to methanogens. Low C/N ratios occur when too much nitrogen is present [30–32]. C/N ratio, along with the other factors, has to be at the optimum value to satisfy the process stability [33]. The suitable C/N ratio for the effective metabolic processes of microbial groups falls within the range of 20–30, which is sufficient to maintain system stability and meet expected energy and nutrient requirements for cell growth [26]. Substrates with high C/N ratios have poor buffering capacity and produce excessive amounts of VFAs during fermentation. In contrast, substrates characterized by low C/N ratios have high buffer capacity and the increased concentration of ammonia in the fermentation process leads to microbial growth inhibition.

The performance of anaerobic digesters can be reduced by various environmental factors, including undesirable pH range, accumulation of ammonia, and VFAs, which inhibit the activity of methanogenic microorganisms. Except for ammonia, other factors, such as sulphide, sodium and potassium, heavy metals, volatile fatty acids, long-chain fatty acids, and hydrogen, can also affect the activity of methanogens. Molecular hydrogen is formed throughout different stages of anaerobic digestion [34,35].

Inhibition can occur due to the lack of balance between the rates of hydrolysis and methanogenesis. A suitable balance between those rates is essential for higher methane production. Rapid methanogenesis is required to prevent accumulation of organic acid lowering pH to an extent that inhibits methanogenesis [36–39].

Some nutrient elements are needed for the growth of methane-forming bacteria. Particular metals comprising nickel, iron, cobalt, and molybdenum are essential for optimal growth and methane production. Trace metals stimulate methanogenic activity. Some metals, including selenium, molybdenum, manganese, aluminum, and boron, have been suggested as additional components in order to achieve a nutrient-rich medium. The addition of metal ion solutions to anaerobic digesters can improve the performance of the AD system [30,40].

The increase of the TS fraction leads to a corresponding decrease in the reactor volume. The OLR is defined as the organic matter flowing into the digester over time, which is expressed as the mass of organic matter over digester volume over time [30,36]. OLR is also defined as a measure of the biological conversion capacity of the AD system. When feeding the system above its sustainable OLR, low biogas yield is obtained. This is caused by the accumulation of the inhibiting substances, such as fatty acids in the digester slurry. Any substrate that can be converted to methane by anaerobic bacteria is referred to as feedstock. The main components of feedstock are carbon, oxygen, nitrogen, hydrogen, and phosphorus, and the microbial cell materials of those elements are reported to be approximately 50, 20, 12, 8, and 2%, respectively. Feedstocks can be a range of different waste materials, from easily degradable wastewater to complex high-solid waste [41].

There is a different optimum pH range for each group of micro-organisms. Methanogenic *Archaea* are very sensitive to pH. The optimum range for them is between 6.5 and 7.2. The fermentative microorganisms are relatively less sensitive and can tolerate a wider range of pH between 4.0 and 8.5. Mainly acetic and butyric acid are produced at a low pH, while acetic and propionic acid are produced at a pH of 8.0. The VFAs produced during the AD process result in a pH reduction. This reduction is normally adjusted by methanogenic *Archaea*, which produce alkalinity in the form of ammonia, carbon dioxide, and bicarbonate [30,36]. The pH of the system is controlled by the CO₂ in the gas phase and the HCO₃-alkalinity of the liquid phase. If the concentration of CO₂ remains constant, the addition of HCO₃-alkalinity can increase the pH of the digester. In order to maintain a stable and well-buffered digestion process, a buffering capacity of 70 meq CaCO₃/L or a molar ratio of at least 1.4:1 of bicarbonate/VFAs is required. However, studies has shown that particularly the stability of the ratio is very significant, and not so much its level. The temperature is also an important environmental factor effecting the physicochemical properties of the substrate. Moreover, it is effective on the growth rate and metabolism of micro-organisms and the population dynamics in the reactor. It is very important that a stable operating temperature is maintained in the digester, since fluctuations in temperature affect the bacteria, particularly the methanogens [36,42].

Apart from the environmental factors, a number of operational factors, including solid retention time (SRT), hydraulic retention time (HRT), digestion mode (in terms of single or multi stage approaches), digester design (being batch or continuous types), and digester mixing also affect the AD performance. SRT and HRT are important design and operating parameters for all anaerobic processes. Reduction of SRT decreases the extent of the reactions, and vice versa. A fraction of the bacterial population is removed each time when the sludge is withdrawn. Therefore, the cell growth must at least compensate the cell removal to maintain a steady state and to prevent process failure. In most cases, SRT of >10 days is desired and SRT of less than 10 days and more than 40 days can adversely affect the digester performance [40,43,44]. To attain optimum performance for the AD, it is essential to maintain proper mixing. Mixing causes near contact between the feed sludge and active biomass, yielding uniformity of a number of environmental parameters, including temperature, substrate concentration, other chemicals, and physical and biological aspects throughout the digester. Mixing also prevents the formation of surface scum layers and sludge deposition on the bottom of the tank [36].

Anaerobic co-digestion (AnCoD), which entails the simultaneous digestion of two or more feedstocks, has been shown to be beneficial for its economic viability, increased methane yields, and its capability to alleviate some of the problems emerging in mono-digestion. These problems, such as imbalanced nutrients, existence of toxic materials, or recalcitrant compounds in the feedstock, have made anaerobic co-digestion of multi-feedstock a popular research area in the enhancement of conventional AD technology. Publications on AnCoD have significantly increased within the last fifteen years, indicating its capability for improving biogas production [7,18,20,26].

Several reviews are available on different aspects of AD processes in general. However, there is no adequate study specifically on reviewing the AnCoD of multi-feedstock. This article is mainly focused on the analysis of microbial populations of AnCoD and the impact of using a multi-stage process in the anaerobic co-digestion process. It aims to provide a review on the influence of adding co-substrates in the anaerobic co-digestion of multiple feedstocks to improve biogas production. Primarily it will discuss the advantages of co-digestion over mono digestion in terms of system stability, bioenergy, and solids reduction. The influence of adding co-substrates on microbial consortia diversity and their synergistic impact on biogas improvement will then be delineated, and lastly the effect of digester mode (i.e., multi-stage versus single stage digestion) on enhancing biogas in the AnCoD process when using multiple feedstocks will be presented.

2. Anaerobic Co-Digestion (AnCoD)

AnCoD involves a simultaneous digestion of two or more substrates. The main goal of anaerobic co-digestion is to increase biogas, mainly biomethane for heat and electricity. As shown in Figure 2, a range of feedstocks can be co-digested at a suitable blend ratio to maintain optimum conditions required for metabolic activity and improved biogas production for heat and electricity. Anaerobic co-digestion has been shown to be a viable option to alleviate the drawbacks of mono-digestion, while enhancing the economic feasibility of the existing AD plants by increasing methane yields [23,31,45].

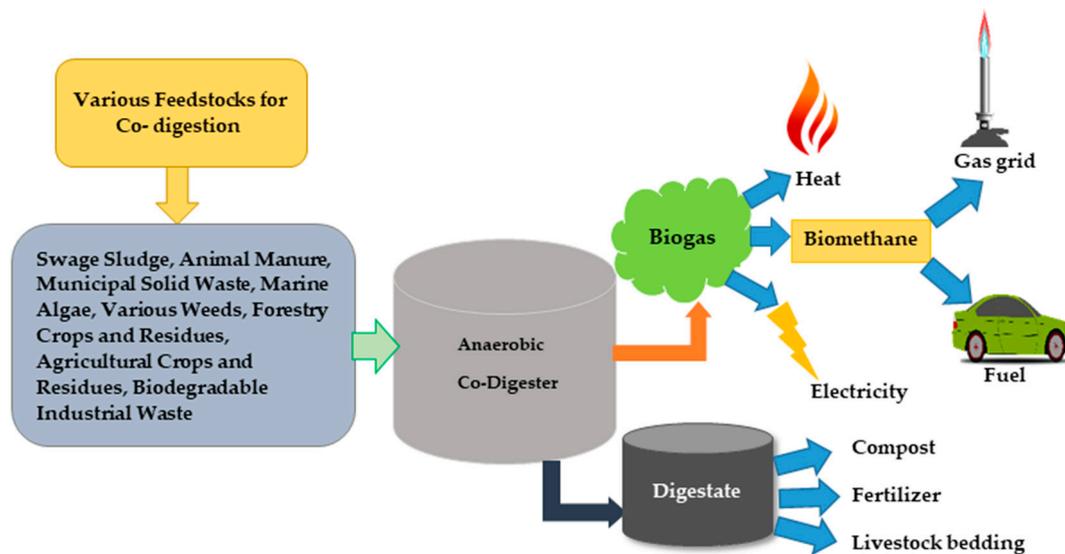


Figure 2. Co-digestion of multi feedstocks for waste reduction and energy recovery.

Various advantages of AnCoD systems are presented in Figure 3. When applying AnCoD, multiple aspects are considered. The cost of transporting the co-substrate from the generation point to the plant seems to be the most common consideration, while the selection of the best co-substrate and blend ratio in order to enhance synergism, dilute disruptive compounds, and optimize methane production and digestate quality are also important considerations that plants evaluate when using AnCoD [45–47].

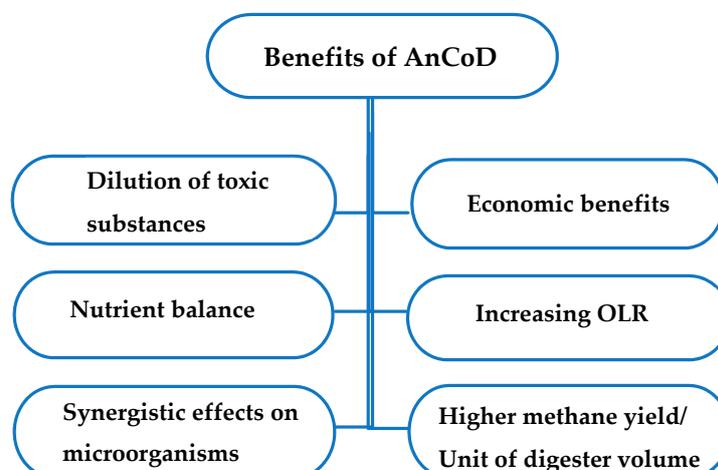


Figure 3. Advantages of anaerobic co-digestion systems.

Co-digestion of different feedstocks with animal manure can increase biogas production from 25% to 400% compared to the mono-digestion of the same substrates. In co-digestion of pig manure with glycerol at a pig manure/glycerol mixing ratio of 24/1 under mesophilic conditions, almost a 400% increase in biogas was observed in comparison with the conventional mono-digestion of pig manure alone [48,49].

Feedstocks characterized by higher C/N ratios (>50), such as rice and wheat straws, corn stalks, seaweed, and algae, can be co-digested by the feedstocks of lower C/N ratios, for instance pig manure, poultry manure, and food and kitchen wastes, to achieve nutrient balance and to avoid the inhibition that leads to system instability and reduced biogas production as a result of an unsuited C/N ratio [26,50–52]. Table 1 shows the possible feedstocks for co-digestion with regard to C/N ratio.

Table 1. Potential feedstocks for co-digestion to balance nutrient with regard to C/N ratio [26,50–52].

Feedstocks with Max C/N Ratio <20	C/N Ratio	Feedstocks with Max C/N Ratio ≤40	C/N Ratio	Feedstocks with C/N Ratio Around or >50	C/N Ratio
TWAS ¹	6–9	OFMSW ³	24	Potatoes	35–60
CSW ²	11	Cow dung	16–25	Oat straw	48–50
Poultry manure	5–15	Horse manure	20–25	Corn stalks/straw	50–56
Pig manure	6–14	Kitchen Waste	25–29	Fallen leaves	50–53
Goat manure	10–17	Peanut shoots/hulls	20–31	Rice straw	51–67
Grass/Grass trimmings	12–16	Slaughterhouse waste	22–37	Seaweed	70–79
Alfalfa	12–17	Mixed food waste	15–32	Algae	75–100
Food Waste	3–17	Waste cereal	16–40	Sugar cane/bagasse	140–150
-	-	Sugar beet/Sugar foliage	35–40	Sawdust	200–500
-	-	Waste cereals	16–40	-	-

Note: ¹ Thickened Waste Activated Sludge, ² Caned Seafood Waste, ³ Organic Fraction of Municipal Solid Wastes.

AnCoD Process and Main Parameters

For the implementation of AnCoD, other than the aforementioned factors that govern the conventional AD process, additional considerations, including the selection of co-substrates and their mixing ratio, should be taken. For instance, mixing materials of high and low C/N ratios, such as organic solid waste mixed with animal manure or sewage, can help achieve optimum C/N ratios (20–30) [26,53].

In order to attain an improved co-digestion process, some precautions and suitable procedures are necessary. There may be requirements for supplementary digester equipment depending on the size of the operation, quality of waste, and characteristics of the wastes to be co-digested. Precautions or supplementary equipment would mainly be required for homogenization and mixing of co-substrates, delivery of waste, prevention of excessive foaming and scum layer formation, and removal of sediment from the digester. Furthermore, proper monitoring parameters should be determined to control and regulate the AnCoD digesters to help maintain an efficient performance when it is under operation. Applying suitable monitoring and control procedures when running the AnCoD process allows for utilization of the full capacity of the system without overload risks. Monitoring can be performed by measuring indirectly the activity of different groups of organisms, for example by measuring the rate of gas production, or the accumulation of intermediates of anaerobic degradation which reflect the existing metabolic status of the active organisms in the system [54].

A number of recommendations in the literature has been proposed, specifying which control parameters should be chosen to be measured for monitoring purposes. Some of the more common ones include pH, alkalinity, VFAs, gas production rate, and the amounts of hydrogen, methane, and carbon dioxide in the gas [55,56].

Partial alkalinity (PA) has been considered as a reliable monitoring parameter [57–59]. The applicability of pH as a process indicator was reported to be intensely dependent on the buffering capacity, making it an unreliable monitoring parameter [55]. It is expected that the selection and

applicability of a specific parameter could not be generalized depending on the individual process configuration and the waste characteristics.

In a study on a full-scale municipal system examining co-digested excess sludge from the municipal wastewater treatment plant with carbohydrate-rich food processing waste, different parameters were assessed for monitoring and control of the system performance. Those parameters included the volume of gas produced, pH, VFAs, and alkalinity. In addition, gas composition and the degradation of organic matter were also measured at steady states and during process changes. Both full-scale and lab-scale experiments were carried out to evaluate the suitability of those parameters. In that research, the digester was run below maximum capacity in order to avoid overload. The only operational limit set for the plant was that the pH should not have been below 6.8. Therefore, the pH was compared with alkalinity, VFA concentration, gas production rate, and the gas composition. Alkalinity was measured as PA. OLR changes were monitored both in the full-scale digester and in the lab-scale models. As indicated by the results of that study, the load's fluctuations were reflected in the pH, PA, and VFA concentrations. At overload condition, all three parameters clearly demonstrated the process imbalance. The VFA concentrations proved to be a better indicator for an overload of the microbial system, although alkalinity and pH showed good monitoring parameters as well. The results indicated that gas-phase parameters demonstrated a slow response to load changes. The response of gas production and gas composition was delayed and significant change was observed only after severe overload [60]. This is in agreement with the results of other studies, which had observed that the change in the gas phase parameters only takes place after well-developed imbalance. For that reason, the gas-phase concentration would not always reflect the actual concentration in the liquid caused by limitations in liquid-to-gas mass transfer [56,61–63].

At a higher OLR of 5.9 KgVS/m³day, the process was shown to be more sensitive to system disturbances. The changes in VFA concentration were not accurately reflected in pH. The increased amounts of VFAs were demonstrated in a lower pH (5.3), because of the low buffering capacity of the process. Nevertheless, the pH was not presented as a reliable means of process monitoring because of possible variation in buffering capacity as a result of variations in substrate composition. Therefore, a process imbalance, causing significant accumulation of VFAs, could be unseen by this buffering effect. Therefore, relying on pH measurements for the process monitoring was not advised and the usage of pH measurements together with measurements of the PA or VFAs was suggested by authors [60].

Some studies have been carried out to evaluate the impact of various factors on AnCoD processes. The main aim of the studies was to assess the influence of those parameters on biogas yield and biogas composition, including biomethane or biohydrogen content. However, no comprehensive guidelines have been compiled so far to standardize the AnCoD systems. This would be firstly due to the complexity of the process caused by the variety of co-substrates and waste composition, and secondly because it has not been broadly implemented at full scale.

In the study conducted by IEA Bioenergy, in addition to the analysis of the ammonia and the volatile fatty acid concentration, identifying the influences of co-substrates on the digester behavior was recommended. It was suggested to maintain a record of the type and amount of separated contaminations in co-digestion. In the case that sterilization is also involved, monitoring the type and the amount of waste streams and the treatment conditions, such as time and temperature, were also considered to be of necessary control parameters. The sampling frequency and methods for analysis required for quality assurance of the end product digestate or compost were also comprehensively defined in that study [64].

Although co-digestion of feedstocks, such as poultry manure and kitchen waste with low C/N ratio with those of higher C/N ratio, such as agricultural waste including rice and wheat straw, is a solution to adjust its ratio to the optimum level, the existence of lignocellulosic material in the agricultural waste caused limitations during AnCoD as a result of long retention time and low biodegradability [65].

Such problems may still demand pretreatment techniques in order to speed up the hydrolysis, which is the rate-limiting step in the anaerobic digestion process. The main purpose of the pre-treatment is to increase the solubilization by the breakdown of the complex substrates, such as lignin in lingo-cellulosic feedstocks or tough cell walls in seaweed biomass, in order to accelerate the hydrolysis rate [66–68].

Rodriguez et al. [69] studied the effect of using co-substrates on methane production in co-digestion of waste paper (WP) with microalgal biomass (MA). Their study was carried out in batch mode and was intended to investigate the influence of the feedstock's mixing ratio (WP/MA), as well as feedstock to inoculum (F/I) ratio. They achieved the highest methane yield of 608 mL CH₄/g VS at the F/I and WP/MA ratios of 0.2 and 50:50, respectively. At this mixing ratio of the feedstocks, the obtained methane yield was more than that of the feedstock's mono-digestion. The maximum increase of 49.58% of the methane yield occurred at the same co-digestion ratio of 50:50 and F/I ratio of 0.4. Their study verified the synergetic effect at the feedstock mixing ratio of 50:50, and all F/I ratios of 0.2, 0.3, and 0.4.

Pretreatment has proved successful at increasing the methane yield of numerous strains of microalgae in the digestion process. Most species of microalgae reduce the digestion rate due to their tough cell wall, consisting of slowly biodegradable material [70].

With the increasing attention on anaerobic co-digestion, a number of research studies have been allotted for co-digestion of various feedstocks and pre-treatment techniques, including mechanical particle size reduction, thermal, chemical, and ultrasonic treatment, enzymatic degradation, among others [71,72]. For instance, mechanical pretreatment with Hollander beater in co-digestion of seaweed biomass with digester sludge increased biogas production by 20% at a ratio of 2:3 of algal pulp to sludge per reactor for 10 min beating time [73]. Sufficient data is available on these topics, although it is beyond the scope of this paper. The existing work aims to review the impact of microbial consortia diversity and digester mode on anaerobic co-digestion.

3. Microbial Diversity in AnCoD

The selection of sludge inocula plays an important role in the effectiveness of biological anaerobic treatment of organic wastes. The analysis of microbial community dynamics has revealed that various waste streams and environmental factors can affect microbial community dynamics in an anaerobic co-digestion process [74–76]. Reportedly, mesophilic anaerobic co-digestion of mixed wastes allows for a better variety of substrates, which in turn supports a wider diversity of bacteria and archaea. More diverse resource input results in more diverse communities and greater metabolic activity [75,77,78]. However, there is limited awareness about the microbial consortia in the anaerobic co-digestion process due to the lack of metabolic data on the microorganisms involved in the process.

A comprehensive understanding of the microbial community is hindered by limitations of conventional molecular technology approaches that are restricted in terms of detecting sophisticated microbial diversity in the environment. Attempts for the analysis of the 16S rRNA gene sequencing have been carried out as an alternative to conventional culture techniques. This method is used to identify and compare microorganisms present within a given sample, and it is a well-established method for studying complex microbial communities or environments that are difficult or impossible to study. The method of 16S rRNA gene-based fingerprints could provide less biased and higher coverage information and can support many unknown details about the mechanism of microbial response to the digester enhancement. An improved understanding of the function and the metabolic role of microorganisms in the anaerobic co-digestion of various pollutants can be obtained by the molecular inventories [79,80]. Some of the results of these studies are presented in Table 2.

Table 2. The influence of using multi-feedstock in various AnCoD systems on microbial consortia diversity and biogas and biomethane increase.

Feedstocks	Microbial Consortia	Digester Mode	HRT	Methane Yield/Biogas Increase %	Ref #
Food, fruit, vegetable + night soil waste	<i>Methanosaeta</i> (predominant methanogen) + hydrogenotrophs	Full scale wet fed-batch	18–20 d C/N 8.6	NA	[75]
Fruit vegetable waste + Food waste	<i>Methanoculleus</i> , <i>Methanosaeta</i> , <i>Methanosarcina</i>	CSTR (mesophilic)	NA ¹	0.49 m ³ CH ₄ /kg VS	[77]
Dairy wastewater + Cattle manure	Uncultured <i>Bacteroidetes</i> , <i>Firmicutes</i> , <i>Synergistetes</i> , <i>Syntrophomonas</i> strains <i>Methanosarcina</i> species	ASBR (mesophilic)	20 d C/N 24.7	biogas produced: 0.87 L/g VS removed	[81]
Cow manure + grass silage	<i>Clostridia</i> , unclassified <i>Bacteria</i> , <i>Bacteroidetes</i>	CSTR mesophilic	20 d	NA	[82]
Cow manure + oat straw	<i>Clostridia</i> , unclassified <i>Bacteria</i> , <i>Bacteroidetes</i> , <i>Deltaproteobacteria</i>	CSTR (mesophilic)	20 d	NA	
Cow manure + sugar beet tops	unclassified <i>Bacteria</i> , <i>Clostridia</i> , <i>Bacteroidetes</i> , <i>Bacilli</i>	CSTR (mesophilic)	20 d	NA	
Food wastewater + WAS	Dominated by <i>Methanothermobacter</i> and <i>Methanosarcina</i>	CSTR (thermophilic)	20 d	Max biogas: 316.11 mL CH ₄ /g COD removed	[83]
STP-OGW + SC-OFMSW	<i>Methanobacterium</i> , <i>Methanoculleus</i> , <i>Methanothermobacter</i> uncultured archaea	Batch (thermophilic)	14.4 d	52% biogas and 36% methane increase	[84]
Sewage sludge + FOG	Dominantly <i>Methanosaeta</i> , and N09	Semi-continuous (mesophilic)	15 d	35% biogas increase	[85]
biodiesel waste glycerin + municipal waste sludge	Dominated by <i>Methanosaeta</i> and <i>Methanomicrobium</i>	Two-stage CSTR (mesophilic)	20 d	100% biogas and 120% methane increase	[86]
Food waste + horse manure	Dominated by <i>Aminobacterium</i> , <i>Clostridium</i> , <i>Proteiniphilum</i> , and <i>Saccharofermentans</i>	compact three-stage	NA	11–23% methane increase	[87]
Food waste + cow manure	<i>Firmicutes</i> , <i>Methanobacterium</i> and <i>Methanosaeta</i>	CSTR mesophilic	20 d	26% methane increase	[88]
	<i>Thermotogae</i> , <i>Firmicutes</i> , <i>Synergistetes</i> and <i>Methanothermobacter</i>	CSTR thermophilic	20 d	methane did not increase by co-digestion	
Food waste + Wheat straw	Dominated by <i>Bacteroidetes</i> and <i>Methanotherrix</i>	CSTR mesophilic	NA	30% biogas increase	[89]
	Dominated by <i>Thermotogae</i> and <i>Methanosarcina</i>	CSTR thermophilic	NA	45% biogas increase	

Note: ¹ Not available.

Microbial Community Structure and Synergy

Organic matter in the AD process is decomposed synergistically by a bacterial consortium producing biogas, including biomethane [90,91]. The process involves at least three functional groups of microorganisms that mainly regulate the mutual metabolic interactions under anaerobic conditions. The first microbial community hydrolyzes complex polymeric substances, such as lipids, cellulose, and protein, to fundamental structural building blocks, such as glucose and amino acids. Subsequently, fermentation of these products to fatty acids, acetate, and hydrogen is proceeded by the second community. Among degradation processes involved in anaerobic digestion, the hydrolysis–acidogenesis process has been shown to be the most important step. Hydrolysis is considered as the rate limiting step and in the case of insufficient buffering capacity, accumulation of volatile fatty acids in the reactor during the acidogenesis step can be difficult to control on a large scale as a result of decreasing pH below the range of 6.4–8, which is suitable for methanogens. The third community develops a methanogenesis process, through which acetate and hydrogen are converted to methane and carbon dioxide. Therefore, microbial communities are vital to a stable and efficient transformation of organic matter to biogas [80].

Studies have shown that co-digestion improves the system stability and methane yield due to synergetic effect. In the co-digestion of sugarcane press mud with food waste, the maximum synergetic effect of 17.5 occurred at a mixing ratio of 80:20. At this mixing ratio, the maximum methane yield of 82.36 mL CH₄/g VS, corresponding to a C/N ratio of 24.46, was obtained, which was 22% and 54% higher than those of sugarcane press mud and food waste mono-digestion, respectively [92].

Co-digestion of alginate-extracted (AEWLJ) and non-extracted (NAEWLJ) waste of *Laminaria japonica* with rice straw (RS) at different mixing ratios was studied in batch mode. The results showed that co-digestion of AEWLJ/RS at 67% mixing ratio produced the highest biogas yield of 247 mL/g VS, being 36% higher than the AEWLJ alone. The synergetic effect improved the total methane yield up to 78% and 88% for combinations of AEWLJ/RS and NAEWLJ/RS, respectively [93].

The general metabolism of microbial consortia involves extracellular polymeric substances (EPS) of sludge aggregates. EPS is partly the result of the microbial metabolism that is affected by the microbial community structure and its activity. Growth conditions control the quantity of EPS, which in turn affects the anaerobic digestibility and biogas production. It is not yet clear how different microorganisms contribute to EPS secretion. A comparative study on the pathways of substrate degradation and the by-products of EPS subfractions could provide supplementary data on long-term impacts of microbial activity on anaerobic co-digestion reactors [94,95].

Monitoring qualitative and quantitative changes in a microbial community structure allows for the evaluation of the influence of the co-substrate on microbial populations contributing to the biogas production. However, there is not sufficient literature on this topic. Some studies have been conducted on the microbial community structure and its influence on anaerobic co-digestion processes. Such studies have been aimed at increasing methane production by co-digestion of different organic-rich waste streams, and they have been mostly developed with a view to the influencing parameters (such as mixing ratio, HRT, temperature, and carbon to nitrogen (C/N) ratio) on the population of methanogenic archaea species. Although they reported the selected values of the parameters in their experiments, they have not evaluated how the change of these parameters influence the microbial population and methane production in co-digestion of the same feedstocks, and there are only few studies on this area from the available literature.

In the co-digestion of cow manure and food waste, the effect of temperature on microbial consortia and methane production was investigated. Both thermophilic and mesophilic reactors operated at a 20-day HRT in CSTR mode. It was indicated that the change of temperature influenced the microbial community structure. The analysis of microbial population showed that the species of *Firmicutes* was dominant bacteria in mesophilic reactor at 37 °C. Both *Methanobacterium* and *Methanosaeta* species of archaeal community were observed in the mesophilic reactor with similar abundance. In thermophilic conditions at 55 °C, the dominant bacteria were *Thermotogae*, *Firmicutes*, and *Synergistetes*. The genus *Methanothermobacter* were the dominant archaea in thermophilic condition in the co-digestion system. The result of this study showed that mesophilic conditions at 37 °C increased methane production by 26%, while it did not enhance production of methane in thermophilic reactors co-digesting food waste with cow manure [88]. In contrast, the result of the study on co-digestion of food waste and wheat straw at different mixing ratios and constant OLR of 3 kg VS/m³d in the lab scale CSTR systems showed that the increase of temperature improved the biogas production rate in thermophilic reactors operating at 55 °C, being 4.9–14.8% higher than that of mesophilic reactors at 35 °C. It was also observed that with the increase of food waste proportions, bacteria belonging to the phylum *Thermotogae* became predominant in thermophilic conditions, while under the mesophilic condition, the predominant bacteria was *Bacteroidetes*. The species of *Methanosarcina* was the major methanogen under the thermophilic condition, while the predominant methanogen was *Methanotherrix* under the mesophilic condition. The optimal proportion of the food waste in co-digestion with wheat straw was observed to be 50 and 90% for thermophilic and mesophilic conditions, respectively [89]. Some of the results of these studies are summarized in Table 2.

It is reported that an even distribution of hydrogenotrophic and acetotrophic methanogen populations in a reactor is indicative of a stable operating condition [96,97]. Yang et al. [85] in 2016 studied the performance of a co-digester for the treatment of sewage sludge with fat, oil, and grease (FOG) using a mesophilic semi-continuous reactor and compared it to that of a mono-digester receiving only sewage sludge. Their study indicated that the secretion of EPS increased by 40% in comparison with the mono-digester and that the improvement in co-digestion performance was stimulated due to the release of EPS. The analysis of the microbial 16S rRNA gene showed the dynamic change of the microbial community through the process. Both the bacterial and archaeal community went through a process with FOG addition, and a large amount of consortia, such as *Methanosaeta* and *N09*, were involved in the process. As compared to sewer sludge mono-digestion, biogas production and TS removal efficiencies increased up to 35% and 26%, respectively. It was

shown that FOG addition resulted in nutrition balance and regulation of microbial composition. Additionally, metabolic activities were stimulated and more EPS were obtained with the progressive addition of FOG. Toumi et al. [81] investigated the microbial community's structures in anaerobic co-digestion of dairy wastewater and cattle manure. A maximum VS reduction of 88.6% and biogas production of 0.87 L/g VS removal were obtained through their research, corresponding to a C/N ratio of 24.7 at HRT of 20 days. The bacterial profile analysis showed a large quantity of uncultured *Bacteroidetes*, *Firmicutes*, and *Synergistetes bacterium*. The Syntrophomonas strains associated with H₂-using bacteria, comprising *Methanospirillum* sp., *Methanosphaera* sp., and *Methanobacterium formicicum*, were observed as well. These syntrophic associations are necessary in anaerobic digestion reactors, allowing for maintenance of low hydrogen partial pressure. On the other hand, high concentrations of VFAs resulting from dairy waste acidogenesis allowed the growth of *Methanosarcina* species. It was indicated that high concentrations of VFAs would result preferentially in the growth of the acetoclastic *Methanosarcina* species. The polymers, which are hydrolyzed into soluble compounds under fermentative condition, are converted to acetate and one-carbon constituents by acidogens and acetogens, and these intermediates in turn can be transformed directly by methanogenic archaea into methane and carbon dioxide [98–100].

The anaerobic digestion process of cellulosic material, including grass silage, oat straw, and sugar beet tops, is a multistep process mediated by bacteria and methanogenic archaea to produce methane. It was found that in anaerobic digestion of cellulytic feedstocks, significant cellulolytic contributions occur due to the existence of species belonging to the order *Clostridiales* [99].

Leclerc et al. [101] identified the diversity of dominant archaea in 44 anaerobic digesters fed with various feedstocks, including pulp and paper effluent, pig slurry, slaughterhouse waste, municipal waste, etc., utilizing single strand conformation polymorphism (SSCP) analysis and sequencing of 16S rDNA. According to their findings, the diverse range of the dominant archaea in the digesters comprised 23 different molecular species, which belong to *Euryarchaeota* phylum of the archaea domain. From the 44 digesters, the most frequent archaeal sequences were close to *Methanoseta concilii* and *Methanobacterium* clade and occurred in 84% and 73% of digesters, respectively. The other sequences lie under the *Methanobacteriales* and the *Methanomicrobiales* families. The paper-type effluent, such as the effluent from paper factories and the pulp and paper industry, demonstrated an effect on the distribution of the archaeal population, whereas no correlation between the nature of the effluent and the distribution of the archaeal community was observed for other types of effluent. For instance, no correlation was observed when using vinasses-type effluent or alcohol effluent as feedstock to the digesters. For the digesters fed with paper-type effluent, archaeal composition showed increased dominance of clone *vadinDC06* and *Methanobacterium formicicum* sequences.

Wang et al. [82] evaluated co-digestion of cow manure with grass silage, oat straw, and sugar beet tops in three continuously stirred tank reactors. The analyses indicated that major microorganism groups present in the three reactors included *Clostridia*, *Unclassified Bacteria*, and *Bacteroidetes*. However, *Bacilli* or *Deltaproteobacteria* groups were unique to the reactors that included sugar beet and straw with cow manure, respectively. *Unclassified Bacteria* dominated in the sugar beet and cow manure co-digester, while *Clostridia* was the dominant group in the straw and grass reactors. The highest amounts of methane yield and VS removal were obtained in the cow manure-grass reactor, while the lowest values were observed in the reactor of co-digested cow manure with oat straw.

Liu et al. [83] studied anaerobic co-digestion of waste activated sludge (WAS) and food wastewater to examine the effects of food wastewater mixing ratio on the populations of methanogenic archaea species and organic matter removal. Microbial analysis showed relatively high abundances of two methanogenic groups, including *Methanothermobacter* and *Methanosarcina*, throughout the process. The changes in the population of methanogenic archaea was correlated with biogas production and organic matter removal, which were intensely affected by organic acid concentration. As reported by Liu et al. [83], Methanogenic archaea population was positively correlated with the increase of food wastewater mixing ratio. The highest biogas production, with a methane production rate of

1423 mL CH₄/L.d, CH₄ content of 68%, CH₄ yield of 316 mL CH₄/g COD removed, and organic matter removal (VS removal of 77%, TCOD removal of 65.5%), was obtained at a food wastewater mixing ratio of 75%. The results indicated that the maximum population of methanogenic archaea occurred at the food wastewater mixing ratio of 75%. The dynamics of the archaeal community and the correlations between environmental factors and methanogenic community structure was investigated by Lin et al. [90]. Their lab-scale continuous flow stirred-tank reactor (CSTR) co-digested fruit and vegetable waste with food waste at different mixture ratios. With the increasing ratio of fruit and vegetable waste to food waste, the community was predominated by *Methanoculleus*, *Methanosaeta*, and *Methanosarcina*. The shift in the methanogenic community was correlated with the composition of acidogenic and methane yield. Different compositions of intermediate metabolites were observed as a result of applying different mixing ratios of the substrates. In a study conducted at Rayong Biogas Plant in Thailand [75], it was examined how the microbial community structure was affected by the different stages of the AnCoD process, and how co-digestion of mixed waste supported different methanogenic and bacterial pathways. The digestion occurred in a three-stage reactor under mesophilic conditions and treated municipal solid waste, including food waste, fruit, and vegetable waste and night soil waste. *Methanosaeta* was found to be the predominant methanogen, suggesting the acetoclastic methanogenesis to be the main pathway. Resource availability by utilizing different waste streams and environmental factors were verified to be the main factors influencing microbial community dynamics in a mesophilic, anaerobic co-digestion. In the first two stages, digestion of mixed wastes supported acetate, lactate-, and butyrate producing bacteria. This is considered to be common for the AD of such waste configurations [77]. The VFAs were degraded further by the methanogens in Stage 3 (the main digester). Methanogenesis was further improved by the increase of pH and resource availability at this stage and the presence of acetate oxidizing bacteria, such as *Arcobacter*-suggested acetate oxidation. According to the authors, these organisms would have been introduced with the night soil waste. There was a possibility that denitrification and methanogenesis occurred simultaneously during digestion, since the abundance of denitrifying bacteria throughout the AD process was observed. It was revealed that *Comamonas denitrificans* were recovered from stages 1 and 2 [75]. A previous study by Osaka et al. in 2006 [102] had found that *Comamonas denitrificans* assimilated acetate under nitrate-reducing conditions in activated sludge. According to Supaphol et al., the increase in the C/N ratio that was observed between stages 1 and 2 would be due to nitrogen removal from the system through denitrification.

The analysis of 16S rRNA proved stage 3 to be the most productive stage, as indicated by the increase in populations of both archaea and bacteria, and also the approximately 50% removal and conversion of VSS to biogas. Razaviarani et al. [86] studied the correlation between the reactor performance and the microbial community dynamics during the AnCoD of different loadings of biodiesel waste glycerin with municipal wastewater sludge in a two-phase digester. The genus *Methanomicrobium* (hydrogenotrophic methanogens) and the genus *Methanosaeta* (acetoclastic methanogens) were found to be the dominant methanogenic populations. They were detected in almost equal sequence abundances in the control reactors, which were fed only with municipal wastewater sludge.

This suggested that both groups of methanogens were responsible for the methane production in the control reactors. As compared to the control reactor for phase 1, the sequence abundance of genus *Methanosaeta* more than doubled, while the population of the genus *Methanomicrobium* (hydrogenotrophic methanogens) decreased by 96% when biodiesel waste glycerin co-digested with municipal wastewater sludge. The biogas production and methane yield increased by 48% and 56%, respectively. The even distribution of hydrogenotrophic and acetotrophic methanogens was suggestive of the stable conditions of the reactor under moderate loading of biodiesel waste glycerin. Microbial analysis demonstrated *Methanosaeta* and *Methanomicrobium* (hydrogenotrophic) to be the methanogenic groups existing with highest diversity during the stable operation with the moderate biodiesel waste glycerin loading. It was observed that *Methanosaeta* sequences were predominant at

the lowest biodiesel waste glycerin loading, while *Methanomicrobium* sequences prevailed at the higher loadings. The environmental variables, including pH, alkalinity, and VFAs, were reported to be the key factors determining the microbial community dynamics. As compared to the control reactor, in phase 2, approximately a 168% increase in the total sequence abundance of archaea for the co-digestion reactor was observed. In the co digestion reactor, the biogas production and methane yield increased by 2.0 and 2.2 times, respectively. A 4.6 and 1.3-times increase in the sequence abundances of the genera *Methanomicrobium* and *Methanosaeta* was obtained by the addition of 1.35% (*v/v*) biodiesel waste glycerin to the feed. These findings indicated that a greater number of hydrogenotrophic methanogens existed in the co-digestion reactor in comparison to the control reactor [86].

The study by Yang et al. [85] also showed that hydrogenotrophic methanogens were the essential prevailing archaeal group during the digestion of synthetic wastes containing glycerol.

The growth of many more hydrogenotrophic populations than acetoclastic methanogens in the co-digestion reactor in phase 2 was observed. As reported elsewhere [86,103], this is assumed to be due to degradation of propionic acid, the major product of glycerol degradation, which is accelerated in the presence of H₂-utilizing methanogens. Additionally, the production of methane from propionate degradation is thermodynamically feasible only when the H₂ partial pressure is maintained between 0.1 and 10.1 Pa by H₂-utilizing methanogens. The reduction of CO₂ by H₂ via the hydrogenotrophic pathway is more energetically favorable than acetoclastic reactions. Martín-González et al. [84] found that thermophilic conditions were superior to mesophilic conditions for the enhancement of AnCoD along with the use of sewage treatment plant fat, oil, and grease waste (STP-FOGW) as co-substrates in co-digestion of sources collected from organic fraction of municipal solid waste (SC-OFMSW). Monitoring of the microbial structure demonstrated that the bacterial profiles were clustered in two separate groups, before and after the extended contact with FOG waste, whereas the archaeal community structure remained relatively constant throughout the operation. The bacterial population structure showed a dynamic change determined to be due to introducing FOG residues to the reactor. The low diversity of the archaeal community that was observed in their study was in agreement with another study by Weiss et al. in 2008 [104] that showed a lower diversity in thermophilic anaerobic reactors as compared to mesophilic ones. The stable reactor performance during their whole experiment would be due to the development of a main consortium acclimated to this sort of complex residue. Furthermore, indications of inhibition, including VFA accumulation or decrease in biogas production, were not observed after feeding FOG waste to the reactor. During the co-digestion period, a 52% increase in biogas production from 11 to 17 L/d, and 36% in methane yield from 0.36 to 0.49 L CH₄/g VS_{added}, were achieved.

It is evident that both communities of methanogens, including bacteria and archaea, are found in AnCoD systems. However, *Methanosaeta* species, which belong to the archaeal consortium, are the most abundant population of methanogens that are present in the majority of the AnCoD systems. *Methanosaeta* species are very active methanogens and are able to produce a large amount of methane. The species of the bacterial community, such as *Clostridia*, are commonly found in feedstocks, such as cattle manure, grass silage, and sugar beet, which can be introduced to the system, which utilizes them as co-substrates. In bioreactors, including AnCoD systems, characterization of microbial consortia is useful for understanding and optimization of the processes in order to increase the methane yield. Production of biomethane occurs through syntrophic metabolism between both communities. The key pathway for producing methane through the syntrophic metabolism includes hydrogen transfer between both methanogenic consortia of bacteria and archaea.

4. Digester Stage

In general, anaerobic digesters can be configured as one-stage, two-stage, or multi-stage reactors, in which the hydrolysis/acidogenesis and acetogenesis/methanogenesis steps occur in either the same or separated digesters (Figure 4). Separating the digesters makes the process easier to control, and makes it possible to separately optimize the operational and environmental conditions for

hydrolysis/acidogenesis and methanogenesis processes in order to enhance the overall reaction rate and biogas yield [105,106].

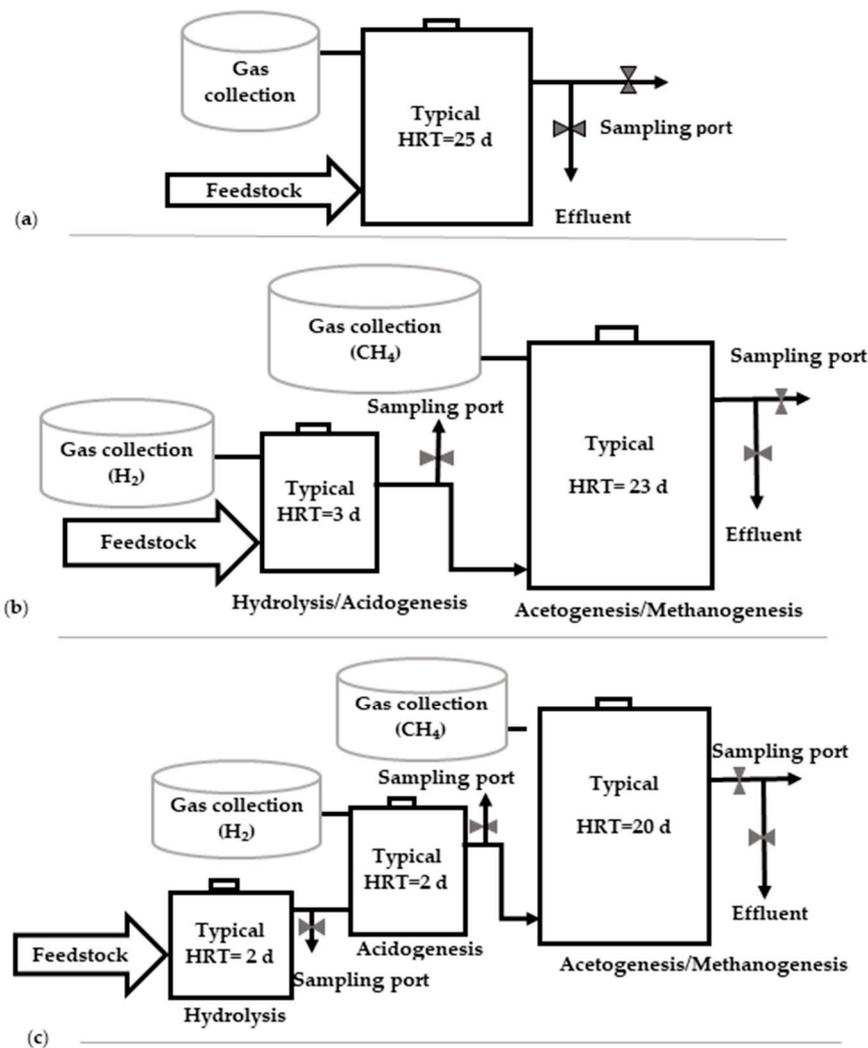


Figure 4. Types of digester configuration: (a) Single-stage; (b) two-stage; and (c) three-stage digester.

Fluctuations in organic loading rate, heterogeneity of wastes, or the presence of excessive inhibitors can lead to instability of the process, and multi-stage systems have been shown to be more stable as compared to single-stage ones. Two or multi-stage systems allow for the selection and enrichment of different types of microorganisms in each digester, which results in extending the possibility of processing different biomass constituents, improving substrate conversion, enhancing the chemical oxygen demand (COD) reduction, and increasing energy recovery. Although multi-stage digesters are associated with greater construction and maintenance costs, multi-stage digesters provide higher performances as compared to single-stage systems [107–109]. Using a two-stage digestion, controlled acidogenesis in the first digester helps maintain a high soluble feed to the second stage, which subsequently enhances the biogas production [110].

In the two-stage anaerobic digestion systems, acid fermentation and methanogenesis are separated into two reactors in order to optimize reactor conditions for the different groups of microorganisms. The acidogenic stage is typically operated at a low HRT in the range of 2 to 3 days and a pH of between 5 and 6, while the second stage, methanogenesis, is typically operated with a HRT of 20 to 30 days and a pH between 6 and 8, facilitating the development of slow-growing methanogenic

archaea. Moreover, the acidogenesis phase allows for long chain fatty acid (LCFA) saturation and degradation [30,38].

Owing to the bent molecular structure of unsaturated LCFAs because of the existence of double bonds, they have a greater cover area of 339 cell walls per molecule as compared to saturated LCFAs. As a result, unsaturated LCFAs have demonstrated stronger inhibitory effects in comparison with saturated LCFAs [111–113], as such transformation of unsaturated to saturated LCFAs is beneficial. In addition, LCFA saturation is necessary for the oxidative breakdown of fatty acid molecules and formation of acetic acid [105,114].

The outcome of a study using a two-stage AD system treating a synthetic fat-containing wastewater comprised of a glucose and LCFA mixture revealed that 19% of LCFAs were degraded and 12% of unsaturated LCFAs were saturated in the acidogenic phase [38]. The acidogenic phase can also convert the unsaturated LCFAs to palmitic acid, which reduces the lipid inhibition of methanogenesis in the second stage [115]. Food waste, which composes a large portion of the organic fraction of OFMSW, contains a substantial amount of organic soluble compounds, which can be simply converted to VFAs.

Therefore, it can be an ideal substrate for biogas production. Nevertheless, formation of excessive amount of VFAs at initial digestion stages can result in a remarkable pH reduction and subsequent methanogenesis inhibition. Utilization of two-stage anaerobic digestion systems for food waste has shown to be an effective solution for the pH inhibition of one stage systems [116–121]. Most of the studies on anaerobic co-digestion processes have aimed to evaluate the digester's performance and optimal operating conditions for a particular type of waste.

In the study conducted by Razaviarani et al., in the co-digestion of municipal wastewater sludge with biodiesel waste glycerin in a mesophilic two-stage CSTR reactor, the addition of biodiesel waste glycerin to the municipal wastewater sludge increased the biogas and methane yield compared to the control reactors in each phase. They observed that the biogas production and methane yield increased to 12.2 L/d and 0.53 L/gVSadded, corresponding to 48% and 56% increases in biogas production and methane yield, respectively, compared to the control reactor in phase 1. In phase 2, the addition of BWG to the feed increased biogas and methane yield by 100% and 121%, respectively, compared to the control reactor that digested only municipal wastewater sludge [86].

Lafitte-Trouque et al. found their two-stage thermophilic/mesophilic AnCoD system to be effective for the co-digestion of sewage sludge and confectionery waste. The system with the second digester operating at a HRT of 12 days provided the best performance in terms of stability, VS reductions, and specific methane yield (corresponding to an average 82% methane in the gas composition). However, a HRT of 8 days in the second stage digester was not able to assimilate high concentrations of volatile acid and low pH from the first digester. This was related to the insufficient retention time for maintaining a substantial methanogenic population. In a single-stage digester, a HRT of less than 20 days may cause methanogens to be washed out of the digester. Therefore, HRT is one of the important design parameters for the single-phase operations [122].

The study by Ratanatamskul et al. [123] was conducted at pilot-scale on the development of an energy recovery system using a novel prototype two-stage anaerobic digester. Their system co-digested food waste with sewage sludge from a high-rise building for on-site biogas production. The food waste to sewage sludge mixing ratio of 7:1 by weight was selected as the optimal mixing ratio according to the result of lab experiments, and different HRTs were applied to evaluate the effect of HRT on the digester performance. The amount of biogas increased from 1209.17 to 1704.59 L/d with an increase of HRT from 16 to 24 days. The optimal methane production of 825 L/d occurred at an HRT of 24 days, corresponding to 64% methane content in the produced biogas.

Although the advantages of two-stage over single-stage digestion systems are addressed in the literature [119,124–128], there is a lack of adequate research available in terms of comparison between the performances of single, two, or multi-phase co-digestion systems. Kim et al. [129] developed a two-stage system comprised of a continuously stirred tank reactor for acidogenesis and

a methanogenic upflow bed reactor for the treatment of a high lipid wastewater from a milk and ice cream factory, co-digested with slaughter house wastewater. They obtained a 1.2-times increase in the COD removal, 1.9-times increase in lipids removal, and 1.4-times increase in the methane production compared to the single phase system.

In contrast to the result of that study, no significant increase in the overall energy recovery was attained by Schievano et al. [130] using a two-stage digester co-digesting swine manure and market biowaste in comparison with a single-stage one. Volumetric biogas productions were found to be 1.00 and 0.79 Ndm³/L reactor d for the single-stage and the two-stage systems, respectively, even though the average biogas methane content of the two-stage system showed a 25% increase over that of the single-stage digester. The accumulation of undegraded intermediate metabolites, such as volatile fatty acids, ketones, amines, amino acids, and phenols, was believed to be responsible for the reduced efficiency of the two stage digester. It was concluded that although the two-stage system could be capable of a higher bioenergy production, certain incompetent fermentative pathways may lead to formation of recalcitrant and toxic metabolites.

Hidalgo et al. [131] compared a single-phase with a two-phase reactor for the co-digestion of residues from the used vegetable oil processing industry and pig manure. The maximum methane production of 1.06 m³ CH₄/kg VS removed in the single-stage digestion corresponding to a methane production of 0.69 m³ CH₄/kg VS removal (65% CH₄) was obtained at the end of first 50-day operational period. The average biogas productions of 0.46 and 0.33 m³/kg VS removal were observed for the second and third operational period with methane productions of 0.30 (65.5% CH₄) and 0.22 (66% CH₄) m³ CH₄/kg VS removed, respectively.

The two-phase anaerobic digestion improved VS removal efficiencies and process stability in comparison with the single-phase reactor. Although the single-stage system produced more biogas, a higher methane content in produced biogas was obtained by the two-phase system and the latter was deemed to be more beneficial. Table 3 summarizes the results attained by a number of studies conducted on single and two-stage AnCoD systems.

Table 3. Comparison of single-stage and two-stage digestion in AnCoD systems.

Digester Mode	Feedstocks	Mixing Ratio	HRT	Biogas/Methane Content	Ref #
Single-stage (CSTR, mesophilic)	Sewage sludge + confectionery waste	NA ¹	20 d	Methane yield: 0.36-0.28 m ³ /kg VS applied (76–82% methane) ² Methane yield: 0.3–0.34 m ³ /kg VS applied (66–76% methane) ²	[113]
Two-stage (CSTR, thermophilic/mesophilic)			12 d		
Single stage (plug flow)	Food waste + sewage sludge	7:1 (weight)	24 d 16 d	Biogas production: 1704.59 ± 52.12 L/d 1209.17 ± 48.44 L/d	[114]
Single-stage (UASB)	Slaughter house + milk wastewater	NA	2.14 d	40% Methane increase by two-stage reactor	[120]
Two-stage (CSTR/UASB)			2.9 d		
Single-stage (CSTR, thermophilic)	Market biowaste + swine manure	1:4 (weight)	25 d	0.55 dm ³ /L digester d	[121]
Two-stage (CSTR, thermophilic)			3/22 d	0.54 dm ³ /L digester d	
Single-stage (Batch)	Oil processing wastewater + pig manure	1:3 (weight)	20 d	Average biogas: 0.33 m ³ /kgVS removed, (0.66% methane)	[122]
Two-stage (Batch)			2/18 d	Average biogas: 0.4 m ³ /kgVS removed, (0.67% methane)	
Single-stage	Food waste/horse manure	NA	20 d	45.4 L cumulative methane production	[123]
Two-stage			4/16 d	50.7 L cumulative methane	
Three-stage			2/2/16	55.7 L cumulative methane production	

Note: ¹ Not Available, ² Numbers are mean values after 70-day period of phase 1 and phase 2, respectively.

The results obtained by applying a novel compact three-stage anaerobic digester in co-digestion of food waste and horse manure verified the advantages of the three-phase digester over single and two-stage ones as controls. By using three compartments in the three-stage anaerobic digester, three separated functional zones hydrolysis, acidogenesis, and methanogenesis were created. This configuration significantly accelerated the solubilization of solid organic matters and the formation

of volatile fatty acids leading to an increase of 11 and 23% in methane yield in the two-stage and three-stage digesters in comparison with the single-stage one, respectively. The analysis of 16 S rDNA showed that different microbial communities comprising hydrolyzing bacteria, acidogenic bacteria, and methanogenic archaea were selectively enriched in the three separate reactors of the three-stage digester. *Lactobacillaceae* and *Pseudomonadaceae* were found to be the two predominant hydrolyzing bacteria in the hydrolysis digester. In comparison, the dominant species in acidogenic stage were *Porphyromonadaceae* and *Enterobacteriaceae*, corresponding to almost 70% of the total bacteria [87].

The members of *Lactobacillaceae* are able to ferment carbohydrates to lactate and other by-products, such as acetate, ethanol, formate and succinate, while *Pseudomonadaceae* are able to oxidize carbohydrates and breakdown aromatic rings, and to convert sugars into their biomic acids. It was concluded that the enrichment of these two groups of bacteria contributed to the decomposition and solubilization of food waste and horse manure in the hydrolytic stage. In addition, *Porphyromonadaceae* have the ability to degrade complex carbohydrates and proteinaceous constituents and catalyze the VFA production. It was indicated that its enrichment was linked to the increase of NH_4^+ and VFA concentrations in the acidogenic stage. Methanogenic species were not identified in the hydrolytic and acidogenic stages. However, they were enriched in the methanogenic stage. The abundance of the methanogenic archaea was increased by 0.8 and 1.28 times in the two-stage and the three-stage digesters compared to the single stage one, respectively [87].

5. Conclusions

The current review indicates that implementation of co-digestion mode as compared to mono-digested systems and optimization of digestion performance through the selection of microbial consortia can play prominent roles in the improvement of the overall efficiency of the treatment process and biogas yield, which would be associated with reduced operating costs. This, in turn, results in increased value-added products and reduced environmental footprint and supports local and national economies.

Anaerobic co-digestion offers several benefits over mono-digestion. Using co-substrates enhances performance of the digester and subsequently more biogas production is attained. Additionally, economic benefits can be obtained from the gate fees for waste handling through large scale centralized co-digesting facilities. However, sustainable operation of AnCoD demands appropriate implementation of this process. Overall, the implementation of large scale anaerobic co-digestion systems can lead to the goals of integrated waste management, addressing waste reduction and utilization of renewable energy.

Biomethane is produced via an interaction between archaea and bacteria communities. Analyses of gene sequences can provide insight into anaerobic co-digestion processes and the control strategies. Identifying a correlation between the type of feedstocks and the abundance of the existing species of microbial consortia can be utilized for optimizing the AnCoD systems through the proper selection of the feedstocks and their combination ratio. This could be particularly helpful in controlling multi-stage systems, as the different microbial communities, including hydrolyzing bacteria, acidogenic bacteria, and methanogenic archaea, could be selectively enriched in the three separate reactors in each stage. More studies are required to investigate the microbial consortia in co-digestion of various feedstocks. The advantage of multi-stage over one-stage digesters also requires further investigation and studies.

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References

1. Cheng, H.; Hu, Y. Municipal solid waste (MSW) as a renewable source of energy: Current and future practices in China. *Bioresour. Technol.* **2010**, *101*, 3816–3824. [[CrossRef](#)] [[PubMed](#)]
2. Poggio, A.; Grieco, E. Influence of flue gas cleaning system on the energetic efficiency and on the economic performance of a WTE plant. *Waste Manag.* **2010**, *30*, 1355–1361. [[CrossRef](#)] [[PubMed](#)]
3. Bujak, J.; Sitarz, P.; Jasiewicz, P. Fuel consumption in the thermal treatment of low-calorific industrial food processing waste. *Appl. Energy* **2018**, *221*, 139–147. [[CrossRef](#)]
4. Appels, L.; Lauwers, J.; Degrve, J.; Helsen, L.; Lievens, B.; Willems, K.; Van Impe, J.; Dewil, R. Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renew. Sustain. Energy Rev.* **2011**, *15*, 4295–4301. [[CrossRef](#)]
5. Di Maria, F.; Sisani, F.; Contini, S. Are EU waste-to-energy technologies effective for exploiting the energy in bio-waste? *Appl. Energy* **2018**, *230*, 1557–1572. [[CrossRef](#)]
6. Cornelissen, S.; Koper, M.; Deng, Y.Y. The role of bioenergy in a fully sustainable global energy system. *Biomass Bioenergy* **2012**, *41*, 21–33. [[CrossRef](#)]
7. Kwietniewska, E.; Tys, J. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renew. Sustain. Energy Rev.* **2014**, *34*, 491–500. [[CrossRef](#)]
8. Feng, Z.; Yang, M.; Kumfer, W.; Zhang, W.; Du, Y.; Bai, H. Effect of longitudinal slope of urban underpass tunnels on drivers' heart rate and speed: A study based on a real vehicle experiment. *Tunn. Undergr. Space Technol.* **2018**, *81*, 525–533. [[CrossRef](#)]
9. Guo, M.; Song, W.; Buhain, J. Bioenergy and biofuels: History, status, and perspective. *Renew. Sustain. Energy Rev.* **2015**, *42*, 712–725. [[CrossRef](#)]
10. Schuetzle, D.; Schuetzle, R.; Kent Hoekman, S.; Zielinska, B. The effect of oxygen on formation of syngas contaminants during the thermochemical conversion of biomass. *Int. J. Energy Environ. Eng.* **2015**, *6*, 405–417. [[CrossRef](#)]
11. Di Maria, F.; Barratta, M.; Bianconi, F.; Placidi, P.; Passeri, D. Solid anaerobic digestion batch with liquid digestate recirculation and wet anaerobic digestion of organic waste: Comparison of system performances and identification of microbial guilds. *Waste Manag.* **2017**, *59*, 172–180. [[CrossRef](#)]
12. Mathes, P.G.; Pollard-Durodola, S.D.; Ca'rdenas-Hagan, E.; Linan-Thompson, S.; Vaughn, S. Teaching struggling readers who are native Spanish speakers: What do we know? *Lang. Speech Hear. Serv. Sch.* **2007**, *38*, 260. [[CrossRef](#)]
13. European Commission. *Best Available Techniques in the Slaughterhouses and Animal By-Products Industries*; European Commission: Brussels, Belgium, 2005; 855p.
14. Martinez, E.; Marcos, A.; Al-Kassir, A.; Jaramillo, M.A.; Mohamad, A.A. Mathematical model of a laboratory-scale plant for slaughterhouse effluents biodigestion for biogas production. *Appl. Energy* **2012**, *95*, 210–219. [[CrossRef](#)]
15. Brennan, L.; Owende, P. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.* **2010**, *14*, 557–577. [[CrossRef](#)]
16. Khalid, A.; Arshad, M.; Anjum, M.; Mahmood, T.; Dawson, L. The anaerobic digestion of solid organic waste. *Waste Manag.* **2011**, *31*, 1737–1744. [[CrossRef](#)] [[PubMed](#)]
17. Cardon, P.V. The Food and Agriculture Organization of the United Nations. *Science* **1953**, *118*, 3. [[CrossRef](#)]
18. Gelegenis, J.; Georgakakis, D.; Angelidaki, I.; Christopoulou, N.; Goumenaki, M. Optimization of biogas production from olive-oil mill wastewater, by codigesting with diluted poultry-manure. *Appl. Energy* **2007**, *84*, 646–663. [[CrossRef](#)]
19. Hilkiiah Igoni, A.; Ayotamuno, M.J.; Eze, C.L.; Ogaji, S.O.T.; Probert, S.D. Designs of anaerobic digesters for producing biogas from municipal solid-waste. *Appl. Energy* **2008**, *85*, 430–438. [[CrossRef](#)]
20. Karagiannidis, A.; Perkoulidis, G. A multi-criteria ranking of different technologies for the anaerobic digestion for energy recovery of the organic fraction of municipal solid wastes. *Bioresour. Technol.* **2009**, *100*, 2355–2360. [[CrossRef](#)] [[PubMed](#)]
21. Ward, A.J.; Hobbs, P.J.; Holliman, P.J.; Jones, D.L. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.* **2008**, *99*, 7928–7940. [[CrossRef](#)] [[PubMed](#)]
22. Bordoloi, R.; Lilly, S.J.; Kacprzak, G.G.; Churchill, C.W. Modeling the distribution of Mg II absorbers around galaxies using background galaxies and quasars. *Astrophys. J.* **2014**, *784*, 1–13. [[CrossRef](#)]

23. Bayr, S.; Ojanperä, M.; Kaparaju, P.; Rintala, J. Long-term thermophilic mono-digestion of rendering wastes and co-digestion with potato pulp. *Waste Manag.* **2014**, *34*, 1853–1859. [CrossRef] [PubMed]
24. Mata-Alvarez, J.; Dosta, J.; Macé, S.; Astals, S. Codigestion of solid wastes: A review of its uses and perspectives including modeling. *Crit. Rev. Biotechnol.* **2011**, *31*, 99–111. [CrossRef]
25. Silvestre, G.; Illa, J.; Fernández, B.; Bonmatí, A. Thermophilic anaerobic co-digestion of sewage sludge with grease waste: Effect of long chain fatty acids in the methane yield and its dewatering properties. *Appl. Energy* **2014**, *117*, 87–94. [CrossRef]
26. Hagos, K.; Zong, J.; Li, D.; Liu, C.; Lu, X. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renew. Sustain. Energy Rev.* **2017**, *76*, 1485–1496. [CrossRef]
27. Vindis, P.; Stajniko, D.; Lakota, M. Options for Reduction of Maize Silage in Biogas Plant Drazenci. In *DAAAM International Scientific Book*; 2014; Chapter 9; pp. 121–130. Available online: <https://doi.org/10.2507/daaam.scibook.2014.09> (accessed on 20 March 2019).
28. Viotti, P.; Di Genova, P.; Falcioli, F. Numerical analysis of the anaerobic co-digestion of the organic fraction from municipal solid waste and wastewater: Prediction of the possible performances at Olmeto plant in Perugia (Italy). *Waste Manag. Res.* **2004**, *22*, 115–128. [CrossRef]
29. Kayhanian, M. Biodegradability of the organic fraction of municipal solid waste in high-solids anaerobic digester. *Waste Manag. Res.* **1995**, *13*, 123–136. [CrossRef]
30. Kangle, K.M.; Kore, V.S.; Kore, S.; Kulkani, G.S. Recent trends in anaerobic codigestion: A review. *Univers. J. Environ. Res.* **2012**, *2*, 210–219.
31. Mata-Alvarez, J.; Macé, S.; Llabrés, P. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresour. Technol.* **2000**, *74*, 3–16. [CrossRef]
32. Anaerobic Digestion: Decision Support Software. Available online: <http://erc.epa.ie/safer/resource?id=48a1566c-c3b5-102a-90c6-0593d266866d> (accessed on 21 March 2019).
33. Montingelli, M.E.; Tedesco, S.; Olabi, A.G. Biogas production from algal biomass: A review. *Renew. Sustain. Energy Rev.* **2015**, *43*, 961–972. [CrossRef]
34. Towey, F. The power of two. *Lancet Respir. Med.* **2013**, *1*, 368. [CrossRef]
35. Heo, N.H.; Park, S.C.; Kang, H. Effects of mixture ratio and hydraulic retention time on single-stage anaerobic co-digestion of food waste and waste activated sludge. *J. Environ. Sci. Health Part A Toxic Hazard. Subst. Environ. Eng.* **2004**, *39*, 1739–1756. [CrossRef]
36. Appels, L.; Baeyens, J.; Degreè, J.; Dewil, R. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energy Combust. Sci.* **2008**, *34*, 755–781. [CrossRef]
37. Logan, B.E. *Environmental Transport Processes*, 2nd ed.; John Wiley & Sons, Inc.: Hoboken, NY, USA, 2012; ISBN 9781118230107.
38. Kim, S.H.; Han, S.K.; Shin, H.S. Kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation and beta-oxidation. *J. Environ. Sci. Health Part A Toxic Hazard. Subst. Environ. Eng.* **2004**, *39*, 1025–1037. [CrossRef]
39. Pouget, E.R.; Hagan, H.; Des Jarlais, D.C. Meta-analysis of hepatitis c seroconversion in relation to shared syringes and drug preparation equipment. *Addiction (Abingd. Engl.)* **2012**, *107*, 1057–1065. [CrossRef]
40. Azbar, N.; Ursillo, P.; Speece, R.E. Effect of process configuration and substrate complexity on the performance of anaerobic processes. *Water Res.* **2001**, *35*, 817–829. [CrossRef]
41. Gerardi, M.H. *The Microbiology of Anaerobic Digesters*; John Wiley & Sons, Inc.: Hoboken, NY, USA, 2003; ISBN 0471206938.
42. Turovskiy, I.S.; Mathai, P.K. *Wastewater Sludge Processing*; John Wiley & Sons, Inc.: Hoboken, NY, USA, 2005; ISBN 0471700541.
43. Zahedi, S.; Rivero, M.; Solera, R.; Perez, M. Mesophilic anaerobic co-digestion of sewage sludge with glycerine: Effect of solids retention time. *Fuel* **2018**, *215*, 285–289. [CrossRef]
44. De La Rubia, M.A.; Perez, M.; Romero, L.I.; Sales, D. Effect of solids retention time (SRT) on pilot scale anaerobic thermophilic sludge digestion. *Process Biochem.* **2006**, *41*, 79–86. [CrossRef]
45. Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M.S.; Fonoll, X.; Peces, M.; Astals, S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew. Sustain. Energy Rev.* **2014**, *36*, 412–427. [CrossRef]
46. Divya, D.; Gopinath, L.R.; Merlin Christy, P. A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renew. Sustain. Energy Rev.* **2015**, *42*, 690–699. [CrossRef]
47. Long, J.H.; Aziz, T.N.; Reyes, F.L.D.L.; Ducoste, J.J. Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Saf. Environ. Prot.* **2012**, *90*, 231–245. [CrossRef]

48. Shah, F.A.; Mahmood, Q.; Rashid, N.; Pervez, A.; Raja, I.A.; Shah, M.M. Co-digestion, pretreatment and digester design for enhanced methanogenesis. *Renew. Sustain. Energy Rev.* **2015**, *42*, 627–642. [[CrossRef](#)]
49. Meiramkulova, K.; Bayanov, A.; Ivanova, T.; Havrland, B.; Kára, J.; Hanzlíková, I. Effect of different compositions on anaerobic co-digestion of cattle manure and agro-industrial by-products. *Agron. Res.* **2018**, *16*, 176–187.
50. Alqaralleh, R.; Kennedy, K.; Delatolla, R.; Sartaj, M. Biogas recovery from hyper-thermophilic anaerobic co-digestion of thickened waste activated sludge, organic fraction of municipal solid waste and fat, oil and grease. *J. Bioremediat. Biodegrad.* **2017**, *8*, 408. [[CrossRef](#)]
51. Panpong, K.; Srisuwan, G.; O-Thong, S.; Kongjan, P. Anaerobic co-digestion of canned seafood wastewater with glycerol waste for enhanced biogas production. *Energy Procedia* **2014**, *52*, 328–336. [[CrossRef](#)]
52. Sosnowski, P.; Wieczorek, A.; Ledakowicz, S. Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Adv. Environ. Res.* **2003**, *7*, 609–616. [[CrossRef](#)]
53. Akyol, Ç.; Ozbayram, E.G.; Ince, O.; Kleinstaubler, S.; Ince, B. Anaerobic co-digestion of cow manure and barley: Effect of cow manure to barley ratio on methane production and digestion stability. *Environ. Prog. Sustain. Energy* **2016**, *35*, 589–595. [[CrossRef](#)]
54. Gujer, W.; Zehnder, A.J.B. Conversion processes in anaerobic digestion. *Water Sci. Technol.* **1983**, *15*, 127–167. [[CrossRef](#)]
55. Ahring, B.K.; Sandberg, M.; Angelidaki, I. Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 559–565. [[CrossRef](#)]
56. Moletta, R.; Escoffier, Y.; Ehlinger, F.; Coudert, J.P.; Leyris, J.P. On-line automatic control system. For monitoring an anaerobic fluidized-bed reactor: Response to organic overload. *Water Sci. Technol.* **1994**, *30*, 11–20. [[CrossRef](#)]
57. Jenkins, S.R.; Morgan, J.M.; Zhang, X. Measuring the usable carbonate alkalinity of operating anaerobic digesters. *Res. J. Water Pollut. Control Fed.* **1991**, *63*, 28–34.
58. Rozzi, A.; Di Pinto, A.C.; Limoni, N.; Tomei, M.C. Start-up and operation of anaerobic digesters with automatic bicarbonate control. *Bioresour. Technol.* **1994**, *48*, 215–219. [[CrossRef](#)]
59. Wilcox, S.J.; Hawkes, D.L.; Hawkes, F.R.; Guwy, A.J. A neural network, based on bicarbonate monitoring, to control anaerobic digestion. *Water Res.* **1995**, *29*, 1465–1470. [[CrossRef](#)]
60. Björnsson, L.; Murto, M.; Mattiasson, B. Evaluation of parameters for monitoring an anaerobic co-digestion process. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 844–849. [[CrossRef](#)]
61. Frigon, J.C.; Guiot, S.R. Impact of liquid-to-gas hydrogen mass transfer on substrate conversion efficiency of an upflow anaerobic sludge bed and filter reactor. *Enzym. Microb. Technol.* **1995**, *17*, 1080–1086. [[CrossRef](#)]
62. Weiland, P.; Rozzi, A. Start-up, operation, monitoring and control of high-rate anaerobic treatment systems: Discusser's report e anaerobic treatment technology for municipal and wastewater. *Water Sci. Technol.* **1991**, *24*, 257–277. [[CrossRef](#)]
63. Pauss, A.; Andre, G.; Perrier, M.; Guiot, S.R. Liquid-to-gas mass transfer in anaerobic processes: Inevitable transfer limitations of methane and hydrogen in the biomethanation process. *Appl. Environ. Microbiol.* **1990**, *56*, 1636–1644.
64. Kim, J.K.; Oh, B.R.; Chun, Y.N.; Kim, S.W. Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. *J. Biosci. Bioeng.* **2006**, *102*, 328–332. [[CrossRef](#)]
65. Saha, M.; Eskicioglu, C.; Marin, J. Microwave, ultrasonic and chemo-mechanical pretreatments for enhancing methane potential of pulp mill wastewater treatment sludge. *Bioresour. Technol.* **2011**, *102*, 7815–7826. [[CrossRef](#)]
66. Esposito, G.; Frunzo, L.; Giordano, A.; Liotta, F.; Panico, A.; Pirozzi, F. Anaerobic co-digestion of organic wastes. *Rev. Environ. Sci. Bio Technol.* **2012**, *11*, 325–341. [[CrossRef](#)]
67. Eastman, J.A.; Ferguson, J.F. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Source J. (Water Pollut. Control Fed. Part I)* **1981**, *53*, 352–366.
68. Noike, T.; Endo, G.; Chang, J.-E.; Yaguchi, J.-I.; Matsumoto, J.-I. Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnol. Bioeng.* **1985**, *27*, 1482–1489. [[CrossRef](#)] [[PubMed](#)]
69. Rodriguez, C.; El-Hassan, Z.; Olabi, A.G. Enhanced methane production from waste paper through anaerobic co-digestion with macroalgae. In Proceedings of the 10th International Conference on Sustainable Energy & Environmental Protection: Bioenergy and Biofuels, Madrid, Spain, 26–27 March 2017; pp. 1–9.

70. Uggetti, E.; Passos, F.; Solé, M.; Garfí, M.; Ferrer, I. Recent achievements in the production of biogas from microalgae. *Waste Biomass Valorization* **2017**, *8*, 129–139. [[CrossRef](#)]
71. Martínez-Valdez, F.; Komilis, D.; Saucedo-Castañeda, G.; Barrera, R.; Sanchez, A. The effect of a short term aerobic pretreatment step on the anaerobic co-digestion of the organic fraction of municipal solid wastes: Liquid extract addition versus solid phase addition. *Waste Biomass Valorization* **2017**, *8*, 1793–1801. [[CrossRef](#)]
72. Arelli, V.; Begum, S.; Anupaju, G.R.; Kurtuti, K.; Shailaja, S. Dry anaerobic co-digestion of food waste and cattle manure: Impact of total solids, substrate ratio and thermal pre treatment on methane yield and quality of biomanure. *Bioresour. Technol.* **2018**, *253*, 273–280. [[CrossRef](#)]
73. Tedesco, S.; Benyounis, K.Y.; Olabi, A.G. Mechanical pretreatment effects on macroalgae-derived biogas production in co-digestion with sludge in Ireland. *Energy* **2013**, *61*, 27–33. [[CrossRef](#)]
74. Lin, J.; Zuo, J.; Ji, R.; Chen, X.; Liu, F.; Wang, K.; Yang, Y. Methanogenic community dynamics in anaerobic co-digestion of fruit and vegetable waste and food waste. *J. Environ. Sci. (China)* **2012**, *24*, 1288–1294. [[CrossRef](#)]
75. Supaphol, S.; Jenkins, S.N.; Intomo, P.; Waite, I.S.; Donnell, A.G.O.; O'Donnell, A.G. Microbial community dynamics in mesophilic anaerobic co-digestion of mixed waste. *Bioresour. Technol.* **2011**, *102*, 4021–4027. [[CrossRef](#)]
76. Bedoya, K.; Coltell-Simon, O.; Cabarcas, F.; Alzate, J.F. Metagenomic assessment of the microbial community and methanogenic pathways in biosolids from a municipal wastewater treatment plant in Medellín, Colombia. *Sci. Total Environ.* **2019**, *648*, 572–581. [[CrossRef](#)]
77. Ike, M.; Inoue, D.; Miyano, T.; Liu, T.T.; Sei, K.; Soda, S.; Kadoshin, S. Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. *Bioresour. Technol.* **2010**, *101*, 3952–3957. [[CrossRef](#)]
78. Kim, E.; Lee, J.; Han, G.; Hwang, S. Comprehensive analysis of microbial communities in full-scale mesophilic and thermophilic anaerobic digesters treating food waste-recycling wastewater. *Bioresour. Technol.* **2018**, *259*, 442–450. [[CrossRef](#)] [[PubMed](#)]
79. Rivière, D.; Desvignes, V.; Pelletier, E.; Chaussonnerie, S.; Guermazi, S.; Weissenbach, J.; Li, T.; Camacho, P.; Sghir, A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* **2009**, *3*, 700–714. [[CrossRef](#)] [[PubMed](#)]
80. Zhang, J.; Wei, Y.; Xiao, W.; Zhou, Z.; Yan, X. Performance and spatial community succession of an anaerobic baffled reactor treating acetone-butanol-ethanol fermentation wastewater. *Bioresour. Technol.* **2011**, *102*, 7407–7414. [[CrossRef](#)]
81. Jihen, T.; Baligh, M.; Amel, F.; Said, N.; Moktar, H.; Maher, G.; Hassib, B. Microbial ecology overview during anaerobic codigestion of dairy wastewater and cattle manure and use in agriculture of obtained bio-fertilisers. *Bioresour. Technol.* **2015**, *198*, 141–149.
82. Wang, H.; Lehtomäki, A.; Tolvanen, K.; Puhakka, J.; Rintala, J. Impact of crop species on bacterial community structure during anaerobic co-digestion of crops and cow manure. *Bioresour. Technol.* **2009**, *100*, 2311–2315. [[CrossRef](#)] [[PubMed](#)]
83. Liu, G.; Zhang, R.; El-Mashad, H.M.; Dong, R. Effect of feed to inoculum ratios on biogas yields of food and green wastes. *Bioresour. Technol.* **2009**, *100*, 5103–5108. [[CrossRef](#)]
84. Martín-González, L.; Castro, R.; Pereira, M.A.; Alves, M.M.; Font, X.; Vicent, T. Thermophilic co-digestion of organic fraction of municipal solid wastes with FOG wastes from a sewage treatment plant: Reactor performance and microbial community monitoring. *Bioresour. Technol.* **2011**, *102*, 4734–4741. [[CrossRef](#)]
85. Yang, Z.H.; Xu, R.; Zheng, Y.; Chen, T.; Zhao, L.J.; Li, M. Characterization of extracellular polymeric substances and microbial diversity in anaerobic co-digestion reactor treated sewage sludge with fat, oil, grease. *Bioresour. Technol.* **2016**, *212*, 164–173. [[CrossRef](#)]
86. Razaviarani, V.; Buchanan, I.D. Anaerobic co-digestion of biodiesel waste glycerin with municipal wastewater sludge: Microbial community structure dynamics and reactor performance. *Bioresour. Technol.* **2015**, *182*, 8–17. [[CrossRef](#)]
87. Zhang, J.; Loh, K.C.; Lee, J.; Wang, C.H.; Dai, Y.; Wah Tong, Y. Three-stage anaerobic co-digestion of food waste and horse manure. *Sci. Rep.* **2017**, *7*, 1269. [[CrossRef](#)]
88. Zamanzadeh, M.; Hagen, L.H.; Svensson, K.; Linjordet, R.; Horn, S.J. Biogas production from food waste via co-digestion and digestion—Effects on performance and microbial ecology. *Sci. Rep.* **2017**, *7*. [[CrossRef](#)] [[PubMed](#)]

89. Shi, X.; Guo, X.; Zuo, J.; Wang, Y.; Zhang, M. A comparative study of thermophilic and mesophilic anaerobic co-digestion of food waste and wheat straw: Process stability and microbial community structure shifts. *Waste Manag.* **2018**, *75*, 261–269. [[CrossRef](#)] [[PubMed](#)]
90. Chandra, R.; Vijay, V.K.; Subbarao, P.M.V.; Khura, T.K. Production of methane from anaerobic digestion of jatropha and pongamia oil cakes. *Appl. Energy* **2012**, *93*, 148–159. [[CrossRef](#)]
91. Liew, L.N.; Shi, J.; Li, Y. Methane production from solid-state anaerobic digestion of lignocellulosic biomass. *Biomass Bioenergy* **2012**, *46*, 125–132. [[CrossRef](#)]
92. Cárdenas-Cleves, L.M.; Marmolejo-Rebellón, L.F.; Torres-Lozada, P. Anaerobic codigestion of sugarcane press mud with food waste: Effects on Hydrolysis stage, methane yield, and synergistic effects. *Int. J. Chem. Eng.* **2018**, *2018*, 9351848. [[CrossRef](#)]
93. Osman, M.M.M.; Shao, X.; Zhao, D.; Basheer, A.K.; Jin, H.; Zhang, Y. Methane Production from alginate-extracted and non-extracted waste of *Laminaria japonica*: Anaerobic mono- and synergetic co-digestion effects on yield. *Sustainability* **2019**, *11*, 1269. [[CrossRef](#)]
94. Sheng, G.P.; Yu, H.Q.; Li, X.Y. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnol. Adv.* **2010**, *28*, 882–894. [[CrossRef](#)] [[PubMed](#)]
95. Yu, Z.; Wen, X.; Xu, M.; Huang, X. Characteristics of extracellular polymeric substances and bacterial communities in an anaerobic membrane bioreactor coupled with online ultrasound equipment. *Bioresour. Technol.* **2012**, *117*, 333–340. [[CrossRef](#)] [[PubMed](#)]
96. Demirel, B.; Scherer, P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. *Rev. Environ. Sci. Biotechnol.* **2008**, *7*, 173–190. [[CrossRef](#)]
97. Kim, S.; Bae, J.; Choi, O.; Ju, D.; Lee, J.; Sung, H.; Park, S.; Sang, B.I.; Um, Y. A pilot scale two-stage anaerobic digester treating food waste leachate (FWL): Performance and microbial structure analysis using pyrosequencing. *Process Biochem.* **2014**, *49*, 301–308. [[CrossRef](#)]
98. Nopharatana, A.; Clarke, W.P.; Pullammanappallil, P.C.; Silvey, P.; Chynoweth, D.P. Evaluation of methanogenic activities during anaerobic digestion of municipal solid waste. *Bioresour. Technol.* **1998**, *64*, 169–174. [[CrossRef](#)]
99. Lynd, L.R.; Weimer, P.J.; van Zyl, W.H.; Pretorius, I.S. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 506–577. [[CrossRef](#)] [[PubMed](#)]
100. Veeken, A.; Hamelers, B. Effect of temperature on hydrolysis rates of selected biowaste components. *Bioresour. Technol.* **1999**, *69*, 249–254. [[CrossRef](#)]
101. Leclerc, M.; Delgènes, J.P.; Godon, J.J. Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. *Environ. Microbiol.* **2004**, *6*, 809–819. [[CrossRef](#)]
102. Osaka, T.; Yoshie, S.; Tsuneda, S.; Hirata, A.; Iwami, N.; Inamori, Y. Identification of acetate- or methanol-assimilating bacteria under nitrate-reducing conditions by stable-isotope probing. *Microb. Ecol.* **2006**, *52*, 253–266. [[CrossRef](#)]
103. Lorenzen, J.M.; Schauerte, C.; Hübner, A.; Kölling, M.; Martino, F.; Scherf, K.; Batkai, S.; Zimmer, K.; Foinquinos, A.; Kaucsar, T.; et al. Osteopontin is indispensable for AP1-mediated angiotensin II-related miR-21 transcription during cardiac fibrosis. *Eur. Heart J.* **2015**, *36*, 2184–2196. [[CrossRef](#)]
104. Weiss, A.; Jérôme, V.; Freitag, R.; Mayer, H.K. Diversity of the resident microbiota in a thermophilic municipal biogas plant. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 163–173. [[CrossRef](#)]
105. Lalman, J.; Bagley, D.M. Effects of C18 long chain fatty acids on glucose, butyrate and hydrogen degradation. *Water Res.* **2002**, *36*, 3307–3313. [[CrossRef](#)]
106. Nathao, C.; Sirisukpoka, U.; Pisutpaisal, N. Production of hydrogen and methane by one and two stage fermentation of food waste. *Int. J. Hydrogen Energy* **2013**, *38*, 15764–15769. [[CrossRef](#)]
107. Azbar, N.; Speece, R.E. Two-phase, two-stage, and single-stage anaerobic process comparison. *J. Environ. Eng.* **2001**, *127*, 240–248. [[CrossRef](#)]
108. Bekkering, J.; Broekhuis, A.A.; Van Gemert, W.J.T. Optimisation of a green gas supply chain—A review. *Bioresour. Technol.* **2010**, *101*, 450–456. [[CrossRef](#)]
109. Cuetos, M.J.; Gómez, X.; Escapa, A.; Morán, A. Evaluation and simultaneous optimization of bio-hydrogen production using 32factorial design and the desirability function. *J. Power Sour.* **2007**, *169*, 131–139. [[CrossRef](#)]
110. Dareioti, M.A.; Kornaros, M. Anaerobic mesophilic co-digestion of ensiled sorghum, cheese whey and liquid cow manure in a two-stage CSTR system: Effect of hydraulic retention time. *Bioresour. Technol.* **2015**, *175*, 553–562. [[CrossRef](#)]

111. Demeyer, D.I.; Henderickx, H.K. The effect of C18 unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta (BBA) Lipids Lipid Metab.* **1967**, *137*, 484–497. [[CrossRef](#)]
112. Koster, I.W.; Cramer, A. Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Appl. Environ. Microbiol.* **1987**, *53*, 403–409. [[PubMed](#)]
113. Thies, E.; Jenkins, T.; Stutzenberger, F. Effects of the detergent Tween 80 on *Thermomonospora curvata*. *World J. Microbiol. Biotechnol.* **1994**, *10*, 657–663. [[CrossRef](#)] [[PubMed](#)]
114. Hanaki, K.; Matsuo, T.; Nagase, M. Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotechnol. Bioeng.* **1981**, *23*, 1591–1610. [[CrossRef](#)]
115. Beccari, M.; Majone, M.; Torrisi, L. Two-reactor system with partial phase separation for anaerobic treatment of olive oil mill effluents. *Water Sci. Technol.* **1998**, *38*, 53–60. [[CrossRef](#)]
116. Dinsdale, R.M.; Premier, G.C.; Hawkes, F.R.; Hawkes, D.L. Two-stage anaerobic co-digestion of waste activated sludge and fruit/vegetable waste using inclined tubular digesters. *Bioresour. Technol.* **2000**, *72*, 159–168. [[CrossRef](#)]
117. Kinnunen, M.; Hilderbrandt, D.; Grimberg, S.; Rogers, S.; Mondal, S. Comparative study of methanogens in one- and two-stage anaerobic digester treating food waste. *Renew. Agric. Food Syst.* **2015**, *30*, 515–523. [[CrossRef](#)]
118. Klocke, M.; Nettmann, E.; Bergmann, I.; Mundt, K.; Souidi, K.; Mumme, J.; Linke, B. Characterization of the methanogenic Archaea within two-phase biogas reactor systems operated with plant biomass. *Syst. Appl. Microbiol.* **2008**, *31*, 190–205. [[CrossRef](#)] [[PubMed](#)]
119. Li, Y.; Park, S.Y.; Zhu, J. Solid-state anaerobic digestion for methane production from organic waste. *Renew. Sustain. Energy Rev.* **2011**, *15*, 821–826. [[CrossRef](#)]
120. Shen, F.; Yuan, H.; Pang, Y.; Chen, S.; Zhu, B.; Zou, D.; Liu, Y.; Ma, J.; Yu, L.; Li, X. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): Single-phase vs. two-phase. *Bioresour. Technol.* **2013**, *144*, 80–85. [[PubMed](#)]
121. Shin, S.G.; Han, G.; Lim, J.; Lee, C.; Hwang, S. A comprehensive microbial insight into two-stage anaerobic digestion of food waste-recycling wastewater. *Water Res.* **2010**, *44*, 4838–4849. [[CrossRef](#)]
122. Lafitte-Trouqué, S.; Forster, C.F. Dual anaerobic co-digestion of sewage sludge and confectionery waste. *Bioresour. Technol.* **2000**, *71*, 77–82. [[CrossRef](#)]
123. Ratanatamskul, C.; Wattanayommanaporn, O.; Yamamoto, K. An on-site prototype two-stage anaerobic digester for co-digestion of food waste and sewage sludge for biogas production from high-rise building. *Int. Biodeterior. Biodegrad.* **2015**, *102*, 143–148. [[CrossRef](#)]
124. Bertin, L.; Grilli, S.; Spagni, A.; Fava, F. Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. *Bioresour. Technol.* **2013**, *128*, 779–783. [[CrossRef](#)]
125. Nasr, N.; Elbeshbishy, E.; Hafez, H.; Nakhla, G.; Hesham El Naggari, M. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. *Bioresour. Technol.* **2012**, *111*, 122–126. [[CrossRef](#)]
126. Park, Y.; Hong, F.; Cheon, J.; Hidaka, T.; Tsuno, H. Comparison of thermophilic anaerobic digestion characteristics between single-phase and two-phase systems for kitchen garbage treatment. *J. Biosci. Bioeng.* **2008**, *105*, 48–54. [[CrossRef](#)]
127. Rao, P.; Arcury, T.A.; Quandt, S.A. Student participation in community-based participatory research to improve migrant and seasonal farmworker environmental health: Issues for success. *J. Environ. Educ.* **2004**, *35*, 3–15. [[CrossRef](#)]
128. Yu, H.W.; Samani, Z.; Hanson, A.; Smith, G. Energy recovery from grass using two-phase anaerobic digestion. *Waste Manag.* **2002**, *22*, 1–5. [[CrossRef](#)]
129. Kim, S.; Shin, H. Enhanced Lipid degradation in an upflow anaerobic sludge blanket reactor by integration with an acidogenic reactor. *Water Environ. Res.* **2010**, *82*, 267–272. [[CrossRef](#)] [[PubMed](#)]

130. Schievano, A.; Tenca, A.; Scaglia, B.; Merlino, G.; Rizzi, A.; Daffonchio, D.; Oberti, R.; Adani, F. Two-stage vs single-stage thermophilic anaerobic digestion: Comparison of energy production and biodegradation efficiencies. *Environ. Sci. Technol.* **2012**, *46*, 8502–8510. [[CrossRef](#)] [[PubMed](#)]
131. Hidalgo, D.; Martín-Marroquín, J.M.; Sastre, E. Single-phase and two-phase anaerobic co-digestion of residues from the treatment process of waste vegetable oil and pig manure. *Bioenergy Res.* **2014**, *7*, 670–680. [[CrossRef](#)]



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