

Review

# A Review of the Role of Critical Parameters in the Design and Operation of Biogas Production Plants

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**Abstract:** Many operating parameters, individually or together, may influence the performance of anaerobic digestion towards biogas or digestate yield and quality maximization. The most preferred method of optimizing an anaerobic digestion plant often relies on how carefully the crucial parameters, such as pH, temperature, organic loading rate, hydraulic retention time, and pressure, are chosen. There is a large amount of literature available on optimization of anaerobic digestion; however, given the continued development and implementation of innovative technologies, together with the introduction of increasingly complex systems, it is necessary to update present knowledge on process parameters and their role on operational ranges and flexibilities in real-life anaerobic digestion system. Accordingly, the present review discusses the importance of the selection of operational parameters in existing technologies and their impact on biogas yield. Notably, the four broad areas of feedstock utilization (substrate, inoculum, codigestion and pretreatment), process condition (pH, temperature, pressure, and reactor design), reactor control (HRT and OLR) and inhibition (Ammonia and VFAs) are covered in this review. In addition, particular emphasis is placed on the most recent innovations that have been or may be implemented in current or future biogas plants.

**Keywords:** anaerobic digestion; biogas; optimization; operating parameters; review

## 1. Introduction

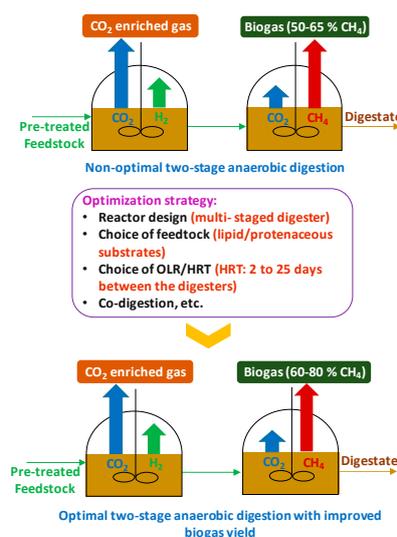
Among all the forms of renewable energy, biomass-based technologies are foreseen to play a critical role in fulfilling the continuously increasing demand for future energy. Because biomass resources are abundant and easily transformable into various forms (solids, liquids, and gases), a vast range of energy applications, i.e., heat, power, chemicals, and liquid biofuels for transportation vehicles, are suited to this source of energy. The global carbon footprint reduction in a long-term future as a result of deploying biomass driven renewable energy technologies is promising. Today, the final energy consumption using biomass-derived fuels have reached 50 EJ globally with potential growth of 150–400 EJ/year in 2100 [1]. Simultaneously, the conversion of fossil fuel vehicles to biofuel vehicles is accelerating rapidly.

Anaerobic digestion (AD), one class of biomass conversion technology, mediated by the syntrophic association of bacteria and archaea in the absence of oxygen [2], has been considered a promising option of treating various types of biomass and wastes, e.g., energy crops, agricultural residues, bio and municipal wastes, industrial wastes (food & beverage), livestock and poultry wastes, sewage, and algae. The products of AD include energy-rich biogas, a potential candidate for diverse downstream conversion

technology, and a liquid residue enriched with macro and micronutrients, suitable as organic fertilizer or soil amendments to agricultural lands. As a result of these manifold output opportunities, AD has been regarded as an excellent biomass conversion alternative, stabilizing utilization of biomass-derived wastes as well as offering the benefit of achieving the circular economy [3]—preserving the concept: ‘reduce’, ‘reuse’, and ‘recycle’.

With the objectives of addressing future energy needs and the global warming reduction, the implementation of commercial and industrial scale biogas plants via AD have been evolving massively in all parts of the world. In Germany alone, the country that has the highest number of biogas plants in the EU, the installed capacity of biogas to electricity plants in the year 2015 exceeded the ~8900 MW capacity [4]. China, one of the biggest biogas producers in Asia, has been experiencing a boom in the biogas generation units predominantly for household-scale applications for the last few decades. However, the considerable investment and public initiatives on innovative measures and technologies have promoted the transformation of many of these household-scale applications to large-scale technologies including enormous growth towards biogas to electricity. Consequently, the biogas to electricity installation capacity in China reached 5500 MW in 2015, which is estimated to escalate up to 30,000 MW by the year 2020 [5]. Among the Nordic countries, the progress in biogas development in Sweden, Denmark, and Finland is reasonably steady [6]. Additionally, Norway has recently made an exemplary stride by commercializing the world’s biggest liquid biogas plant with production capacity estimated to be 25 million Nm<sup>3</sup> of biofuel annually (Biokraft, Skogn, Norway). Once commenced, this will have a direct contribution in reducing 60,000 ton of CO<sub>2</sub> equivalent emission annually, and 25 million L of fossil fuels replacement in transportation application. The biogas integration to the energy sector in other countries like UK, USA, and Australia is also progressing.

Despite the diverse applicability and rapid expansion globally, some factors including process complexity, poor stability, inefficient biodegradability, substrate complexity, and low productivity impede methane production from AD. Numerous ways to overcome operational shortcomings suppressing methane yield have been suggested in previous studies, where the innovative approaches like three-stage digester [7], novel enzyme addition [8] and continuous microbial growth analysis [9] have been developed and implemented successfully. In parallel, optimization of the process performance by manipulating operational variables [10] such as feedstock choice, pretreatment, codigestion, reactor type, temperature, pH and HRT (Hydraulic retention time) [11] have been widely considered. An example of the optimum and conventional AD reactor comparison is shown in Figure 1.



**Figure 1.** Methane content in biogas between conventional and optimum anaerobic digestion (AD).

Despite this, the complex microbiological interactions influencing the performance of operational parameters remain to be extensively explored. In this vein, the overview of the critical parameters and their interdependence to anaerobic digestion efficiency would always be an interesting field of study. This review intends to discuss this relationship and emphasizes on the innovations that may potentially bring significant prospects for future applications.

## 2. Anaerobic Digestion Process and Microbial Communities

Anaerobic digestion offers a valuable option for converting biodegradable feedstock into renewable energy. In this process, the conversion occurs by bacteria and archaea in the absence of oxygen, wherein a series of complex biochemical reactions determine the product output [12]. Generally, the organic part of the biomass is utilized by the microorganism consortia and yielded to methane and carbon dioxide, while the rest is transformed into other minorities [13]. The complete digestion process takes place in four different stages called hydrolysis, acidogenesis, acetogenesis, and methanogenesis [14], which are briefly overviewed in Figure 2.

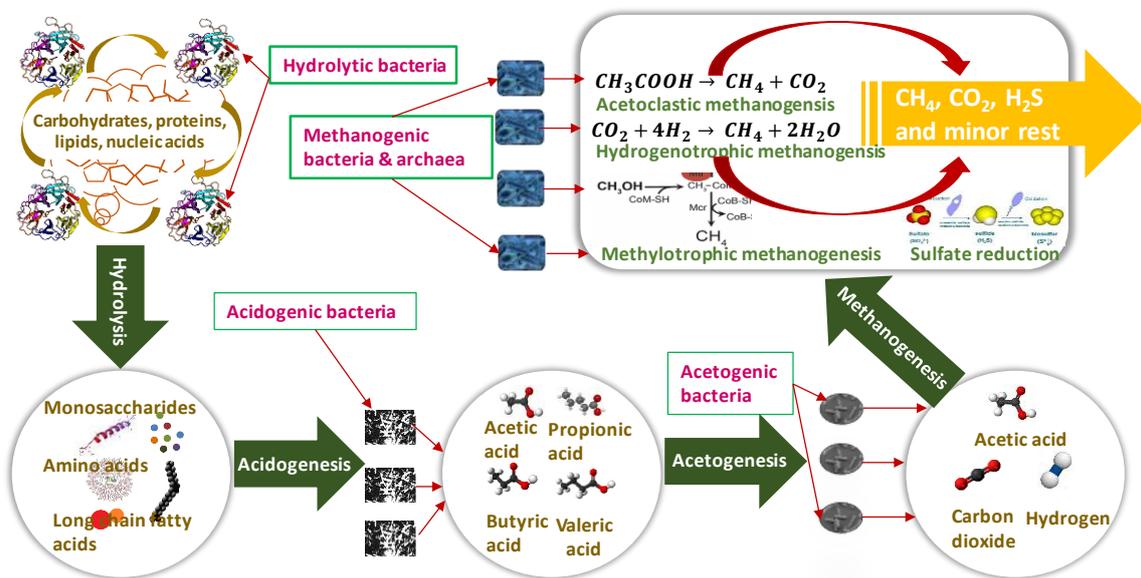


Figure 2. Simple schematic representation of anaerobic digestion steps.

Hydrolysis involves depolymerization of insoluble complex organic hydrocarbons into soluble monomers where the principle substrate compounds (i.e., carbohydrates, lipids, and proteins) are broken down into corresponding low molecular weight monosaccharides, long chain fatty acids, and amino acids that are favorable for bacterial degradation. Hydrolysis is a complex multistep process mediated by extracellular enzymes. The enzymes required for hydrolysis can either be attached to microbial cells or secreted to the solution [15]. Several groups of hydrolytic microorganisms are involved in the degradation of several substrate compositions, where the bacteria Bacteriodes, Clostridium and Staphylococcus are significant drivers [16] (see Table 1).

Table 1. Bacterial groups involved in hydrolysis of different substrate components [16].

Primary Substrate Components	Hydrolyzed Products	Bacterial Group
Carbohydrates	Soluble sugars	<i>Clostridium</i> , <i>Acetovibrio celluliticus</i> , <i>Staphylococcus</i> , <i>Bacteriodes</i>
Lipids	Higher fatty acids or alcohols and glycerol	<i>Clostridium</i> , <i>Staphylococcus</i> , <i>Micrococcus</i>
Proteins	Soluble peptides and amino acids	<i>Clostridium</i> , <i>Proteus vulgaris</i> , <i>Peptococcus</i> , <i>Bacteriodes</i> , <i>Bacillus</i> , <i>Vibrio</i>

In the acidogenesis stage, acidogenic bacteria such as *Lactobacillus*, *Streptococcus*, and *Clostridium* [16] (Table 1) transform hydrolysis products (amino acids and sugars) into volatile fatty acids (VFAs) (acetic acid, butyric acid, and propionic acid), organic acids (succinic acid and lactic acid), ammonia (NH<sub>3</sub>), hydrogen gas (H<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), hydrogen sulfide (H<sub>2</sub>S), and low alcohols [17]. The concentration of hydrogen produced at this stage affects the final product after digestion and the resulting organic matters such as VFAs are not suited for direct conversion to methane by the methanogens.

Hence, the third stage, namely acetogenesis, converts the VFAs, especially acetic acids and butyric acids, into acetate, H<sub>2</sub> and CO<sub>2</sub>. Among the VFAs, 65–95% of methane is directly produced from acetic acid, while propionic acid remains mainly unconverted because its degradation is thermodynamically less favorable (based on the relationship between hydrogen partial pressure and VFA degradation), in comparison to butyric acid [18].

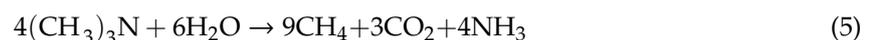
In the final stage, methane is generated by the function of three groups of methanogens, namely acetotrophic, hydrogenotrophic, and methylotrophic [19]. The majority of the methane is produced by acetotrophic methanogens, which transform the acetate (resulting from acetogenesis) into CH<sub>4</sub> and CO<sub>2</sub> [20]. In this process, the principle reaction can be interpreted as shown by Equation (1).



The hydrogenotrophic group converts hydrogen and carbon dioxide into methane through the reactions stated in Equations (2) and (3) [20]. From this route, around 30% of methane may be produced.



Besides the above two groups, some methane can also be produced by the methylotrophic methanogens [19]. Through this pathway, the methyl or trimethylamine component of a given feedstock is transformed into methane following the chemical reactions given by Equations (4) and (5).



### 3. Process Parameters Involved in a Biogas Production Plant

As mentioned earlier, the characteristics of various process parameters impact heavily on the quantity and quality of biogas production. Variables that are broadly relevant to AD include feedstock type, reactor type, pH, temperature, retention time, organic loading, pressure, and inhibitory components. Properties such as inoculum type, codigestion, and pretreatment type are directly linked to feedstock, while temperature, mixing, retention time, organic loading, volatile fatty acids, pH, and pressure are greatly influenced by the type of reactor used and the biochemical interactions involved. The effect of all these parameters on various aspects of anaerobic digestion in light of the present state-of-the-art is widely covered in the following subsections.

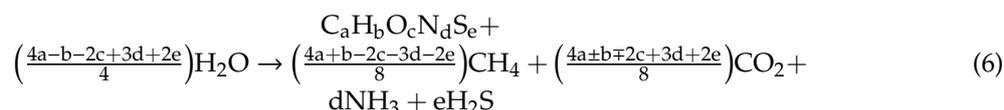
#### 3.1. Feedstock

##### 3.1.1. Substrate

Diverse substrates originated from agricultural wastes, municipal solid wastes, industrial wastes, wastewater, aquatic biomass, and energy crops may be included as feedstock for anaerobic digestion [21]. Extracting biogas from these materials depends on the physical and chemical compositions that favor biological degradation. Not all the substrate components (carbohydrates—cellulose, hemicellulose, and lignin; lipids—fats, oil, and glycerols; and proteins) are readily degradable. For instance, lignin

is highly nondegradable, whereas cellulose breaks down in several weeks; hemicelluloses, fats, and proteins within a few days; and volatile fatty acids, alcohols in several hours [21]. Thus, the choice of appropriate feedstock concerning the amount of energy production, reactor design and waste disposal is essential.

Theoretically, the biogas potential of feedstock constituents can be predicted by employing stoichiometry, as shown in Table 2, or using the empirical formula, proposed by Boyle [22], given in Equation (6).



Among the main components, lipids have the highest methane potential (see Table 2); however, they can cause process problem [23] and inhibition when hydrolyzed into long chain fatty acids (LCFA) and accumulated. Accumulated LCFA due to the adsorption on to anaerobic sludge damages bacterial cell walls, hinders nutrients and metabolic transports, and eventually results in process inhibition [24].

**Table 2.** Stoichiometry of biogas potential determination from various feedstock components [25].

Feedstock	Methane Formation Stoichiometry	Methane Concentration, %
Carbohydrate	$(\text{C}_6\text{H}_{10}\text{O}_5)_n + n\text{H}_2\text{O} \rightarrow 3n\text{CH}_4 + 3n\text{CO}_2$	50
Lipid	$\text{C}_{50}\text{H}_{90}\text{O}_6 + 24.5\text{H}_2\text{O} \rightarrow 34.75\text{CH}_4 + 15.25\text{CO}_2$	69.5
Protein	$\text{C}_{16}\text{H}_{24}\text{O}_5 + 14.5\text{H}_2\text{O} \rightarrow 8.25\text{CH}_4 + 3.75\text{CO}_2 + 4\text{NH}_4^+ + 4\text{HCO}_3^-$	68.8

Food wastes generated from slaughterhouses, food processing industries, and partly from municipal solid waste (MSW) contain a high level of proteins and fats, which, when utilized for AD, influence the production level of ammonia and sulfide. High levels of ammonia are toxic to methanogens and likely to cause process imbalances in terms of pH buffering [26]. Additionally, substrates with a high lipid content may also lead to other process problems associated with the substrate and product transport limitations, sludge floating, foaming, blockage of pipes and pumps, clogging of gas collectors and gas transport system [27]. Nevertheless, due to the high methane potential, lipid-rich feedstocks can offer an enhanced methane production if codigested in combination with substrates like municipal solid wastes [27], sewage sludge [28], paper waste [29], and rice husk [30].

Protein-rich substrates like food waste, fish waste, algae, and energy crops have also been utilized for biogas production via AD. The major problem associated with anaerobic degradation of protein is the development of a high concentration of ammonia that often leads to process instability or inhibition. Some of the process problems related to protein-rich feedstock are suggested to be overcome by means of various approaches where reducing the feedstock particle size [31], keeping operating temperature to mesophilic range [32], increasing HRT [32], ammonia stripping by addition of CaOH, KOH or NaOH [33], and adjusting pH of bioreactor liquid by adding acidic iron and acid [32,34] are commonly used options.

Besides, substrates rich in carbohydrate content, such as lignocellulosic biomass, can be good sources of biogas production. Theoretical biomethane potential of lignocellulosic biomass, such as grass, wheat, straw, and sorghum [35], is relatively high, but due to the high level of lignin, this class of feedstock opposes microbial hydrolysis [36], and hence recalcitrant to anaerobic conversion. A lot of research has suggested pretreatment to maximize utilization of lignocellulosic biomass (see Section 3.1.3).

While lignocellulosic biomass contains a rather high level of lignin, marine feedstock algae (micro and macroalgae) tend to have very low or no lignin, which makes them suitable for AD [37]. Algae as a substrate can circumvent the issue of food vs. fuel, provide an excellent alternative to the

biogas industry, and can offer a synergistic benefit of coproducing various chemicals and value-added products including biogas [38]. So far, the biomethane potential of various algae species has been documented in a number of past studies [39–41]. However, a host of problems are identified, including cell wall resistance for degradation, production of toxic substances, increasing level of pH in case of high C:N ratio algae species, LCFA inhibition [42], and  $\text{NH}_3$  inhibition [37,43]. To overcome part of these challenges, pretreatment, i.e., washing [44], maceration [44], thermal [45], sonic [45], and mechanical [46] treatment along with codigestion [47] may be implemented.

Animal manures are a common source of organic material used as feedstock for AD. Using manures for biogas production reduces anthropogenic greenhouse gas emissions, which would have otherwise released during storage [48]. Depending on factors such as animal species, breed, growth stage, feed, amount, and type of bedding, the biomethane potential (BMP) of animal manure can vary widely. Manure provides essential nutrients (micro and macro) for bacterial proliferation [49], buffer capacity for degradation of low nitrogen substrates, and controlling the level of VFA during AD. Due to its many advantages, most of the anaerobic digesters in Europe [50] are based on animal manure as a substrate. However, the biogas potential of manure is somewhat reduced by its recalcitrant material content, especially the biofibers or the embedded bed materials (e.g., straw). To achieve accelerated conversion of the recalcitrant fraction, manure is often suggested to undergo pretreatment before utilization for biogas production.

### 3.1.2. Inoculum

The choice of right inoculum type in combination with the right temperature is the key to set a desired anaerobic start-up condition [51,52]. Generally, biogas production and the substrate utilization rate in an anaerobic digester show an inverse trend with increasing input of substrate to inoculum (S:I) [53,54]. The usage of too little inoculum can result in incomplete feedstock degradation and process problems associated with VFA accumulation, inhibition and slower methane production rate. For example, depending on the type of inoculum, operating temperature, and the volatile solids content of the substrate, a four-fold increase in S:I ratio could contribute to decreasing as much as 40% of biogas production from AD of cattle manure [55]. Additionally, a higher S:I ratio can lead to longer HRT, resulting in larger reactor working volumes [56]. However, the use of a high amount of inoculum or a low S:I ratio might induce process instability as well as low biogas yield [57]. Thus, a careful compromise is necessary. The effect of the type and amount of inoculum on biogas production from various substrates was studied previously; for example, by Gu et al. [58].

### 3.1.3. Pretreatment

Pretreatment increases feedstock utilization towards AD. Substrates composed of high recalcitrant matter, or not readily biodegradable matter, are considered for pretreatment. The technique used for pretreatment depends on the type of substrate and varies to a wide degree of methods, but, generally, thermal [59], chemical [60], physical/mechanical [61], ultrasound [62], microwave [63], biological [64], and metal addition [11] methods are used to perform pretreatment. The primary feedstock pretreatment categories are shown in Figure 3.

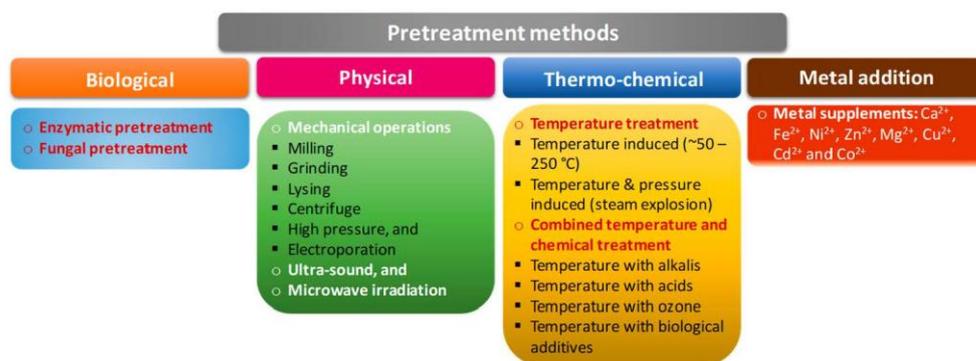


Figure 3. Primary feedstock pretreatment categories—modified from Ref. [65].

Thermal pretreatment occurs at a vast range of temperature, which across various studies spans between 70 °C and 275 °C [59,66–68]. Before anaerobic digestion, the thermal treatment can enhance feedstock hydrolysis resulting in increased anaerobic digestion rate and extent. Substrates like waste activated sludge [66], food waste [69], municipal solid waste [70], agricultural byproducts [71], grass [72], and algae [73] showed a positive effect on methane yield as a result of thermal pretreatment. However, the methane yield from thermally pretreated substrates is not always higher than those that are untreated. Rafique et al. [74] showed no improvement in methane yield from the solid fraction of pig manure at a pretreatment temperature of over 100 °C, while Carrère et al. [75] observed that a pretreatment temperature of 190 °C was found favorable for improved biogas yield from the total liquid and solid fractions of a similar substrate.

Steam explosion is another thermal pretreatment technique mostly applied for treating lignocellulosic biomass. Through steam explosion, the fibers in lignocellulosic biomass open-up and become more accessible for biological degradation. According to the literature [76], biogas yield from lignocellulosic biomass such as *Salix* woodchips can be maximized with the steam explosion method at 210 °C for about 10 min. Steam explosion pretreatment is usually defined by a severity factor that is calculated from the temperature and duration of the process. The relation between the steam explosion severity, duration, and temperature are expressed by Equation (7) below [77]. A similar trend to the *Salix* woodchips was observed for birch wood chips [78], where an approximately two-fold increase in methane yield was achieved compared to untreated woodchips due to the steam explosion with the severity of 4.5 at temperature 220 °C. For agricultural biomass such as wheat straw, the different severity of steam explosion had shown no positive impact on methane yield, but the degradation rate was found to be increased [79]. The severity factor of steam explosion for the majority of feedstocks usually lies within the range of 3.14–3.56 [77].

$$\log R_o = \log \left( t \cdot e^{\left( \frac{T-100}{14.75} \right)} \right) \quad (7)$$

where,

$\log R_o$ : the severity factor as a function of treatment time;

T: the temperature in °C;

t: is the residence time in (min); and

14.75: the activation energy where the process obeys first-order kinetics and the Arrhenius temperature dependence

Apart from thermal pretreatment, thermochemical pretreatment combining heat and chemicals/additives can contribute to enhancing AD productivity by increasing the COD (chemical oxygen demand) solubility [80], reducing feedstock particle size [81], and increasing volatile solid reduction [82]. The chemicals predominantly used for thermochemical pretreatment include alkali [83], acids [84], biological additives [85], and ozone [86]. Acid pretreatment, performed with acids such as HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and H<sub>3</sub>PO<sub>4</sub>, accelerates hemicellulose solubilization into oligomers (i.e., molecules

that consist of a small and specifiable number of monomers; usually less than five), while alkali pretreatment, involving NaOH and Ca(OH)<sub>2</sub>, helps to break lignin together with the solubilization of hemicellulose [65]. Alkali pretreatment (also called saponification) is a commonly adopted technique to help accelerate the hydrolysis of the lipid-like substrate [87]. Compared to acid pretreatment, alkali pretreatment was demonstrated to be more effective in enhancing methane production [65,88], although some issues concerning digestate land application and methanogen toxicity were identified [89]. Despite the certain advantages, the cost associated with the use of additives/chemicals may offset the benefit earned from the revenue of the biogas plants. Therefore, a careful trade-off between cost and biogas yield optimization needs to be practiced. The pros and cons of using a selective list of additives in an anaerobic digestion application are given in Table 3.

**Table 3.** The advantages and disadvantages of using additives in AD [90].

Additive	Element or Compound	Benefit	Adverse Effect
Macro-nutrients	P, N and S	Methane production improvement and enhanced process stability	Methane or biomass inhibition by overdosing
Micro-nutrients, heavy metals	Cu <sup>2+</sup> , Zn <sup>2+</sup> , Cr <sup>3+</sup> , Cd, Ni, Pb <sup>4+</sup> and Hg <sup>2+</sup>	Promoting various enzymatic reactions	Inhibition to the acetogens
Micro-nutrients, light metals	Na <sup>+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> and Al <sup>3+</sup>	Enhancing microbial growth	Restricting production of double cells (Mg <sup>2+</sup> ), Inhibition of acetoclastic methanogens (Na <sup>+</sup> ), Destabilizing buffering system (Ca <sup>2+</sup> ), etc.
Iron	Zero valent iron, Clean scrap, rusty scrap and iron additives	Sulfide fixation, biomass stimulation, etc.	Precipitation and clogging risk
Ash	Bottom ash and fly ash		
Inorganic absorbent materials	MgCl <sub>2</sub> , MgCl <sub>2</sub> .6H <sub>2</sub> O, MgPO <sub>4</sub> .3H <sub>2</sub> O	Ammonia sequestration through struvite formation	
Inorganic nitrogen		Availability of nitrogen as nutrient	Inhibition of methane producing enzymes, VFA accumulation
Nano particles	Ag, Au, Fe, Al <sub>2</sub> O <sub>3</sub> , SiO <sub>2</sub> , TiO <sub>2</sub> , ZnO	Methane production improvement	Inhibition of hydrolytic and methanogenic biomass
Biological additives (bioaugmentation and enzyme)	Compost, <i>C. proteolyticus</i> , SAO co-culture and <i>Methanococcus bourgensis</i> MS2	Increased methanogenic activity, increased hydrolytic activity	Cost, process control
Others	Biochar, activated carbon, sand, zeolite, Ni-Zeo, Co-Zeo, Mg-Zeo, rockwool, membrane, molecular sieve, polyurethane foam and loofah	Biomass immobilization, buffering agents, enhanced VFA degradation	

Unlike thermochemical pretreatment, physical pretreatment does not require the addition of any external compounds but uses physical techniques such as physical operations (milling, grinding, lysing, centrifuge, high pressure, electroporation, ultrasound, and microwave irradiation). Mechanical pretreatment is a widely used physical method that improves bioconversion effectivity, particle densification and distribution, flow properties, porosity, bulk density, and the overall conversion of lignocellulosic biomass into biogas without producing any toxic side streams [91]. With mechanical milling, commonly achieved by the different mills such as attrition mills, ball mills, centrifugal mills, colloid mills, hammer mills, extruders, knife mills, pin mills, and vibratory mills [92], the crystallinity of cellulose, particle size, and the degree of polymerization is reduced and consequently the surface area and digestibility of feedstock are enhanced. Employing milling, such as screw press extrusion, results in an increase of up to 30% in the methane yield from dip litter (a combination of a solid fraction and straw) manure was accomplished [93]. The combination of milling ( $\leq 2$  mm) with fungal pretreatment was observed to achieve a profound methane yield increase of up to 160% [94].

Microwave irradiation is another physical pretreatment option, or a heating method, which directly applies electric and magnetic field components to the molecular structure of the substrate, stimulating physical, chemical, or biological reactions due to the heat and extensive collisions by the

vibrations of the polar molecules and ion movements [95,96]. As a result of microwave radiation, a number of pretreatment benefits can be acquired, which includes increased substrate surface area and accessibility to enzyme attack, decreased polymerization and crystallinity of cellulose and lignin depolymerization [97]. Microwave-assisted pretreatment, using microwave irradiation alone or in combination with various solvents (i.e., water, alkali, acid, ionic liquid, salt, and other organic components), has been studied. With the use of microwave pretreatment alone, a 60% methane yield increase for a continuous reactor was observed [65]. Other evidence suggests that microwave irradiation with a solvent combination is either able to rapidly hydrolyze more sugars [98] or to remove more hemicellulose and lignin from the lignocellulosic substrate [99] compared to the conventional thermal and thermochemical treatment. However, the application of this technique to date is limited to the lab-scale, as technical difficulties to expand it to the industrial scale still exist.

Sonication, involving a sound frequency greater than 20 kHz (ultrasound), can be applied to physical pretreatment of biomass [100]. Brief exposure to ultrasound can cause the thinning of microbial cell walls, resulting in the release of cytoplasm (material within a living cell excluding cell nucleus) and thereby facilitating the intercellular matter to be available for further degradation to CH<sub>4</sub> and CO<sub>2</sub>. Although a large variety of feedstock can be successfully pretreated using ultrasound, until now, this method is widely considered for sewage or waste activated sludge [101], as this material, compared to other substrates, requires a lower sonication time and energy. The past study found that longer sonication time and energy may lead to a lower conversion efficiency of solubilized matter into methane [65]. Based on the literature, the threshold of specific sonication energy ranges between 1000 and 16,000 kJ/kg TS (total solids) and is strongly correlated to substrate solid content, which for sludge was reported to be optimum at 20–30 g/L [65]. With use of sonication, the BMP of waste activated sludge and dairy cattle manure (TS of 5.8%) was improved by 140% [102] and 19% [102], respectively. With high TS substrates, such as mixed sludge and MSW (~9% TS), a very high specific energy requirement of about 90,000 kJ/kgTS resulted in an increase of 24% in the methane yield [103]. Sonication of high solid substrates also caused high cavitation, increased dewaterability and disturbance to the homogeneity of acoustic waves [65]. However, the energy applied to treat high TS (9%) sludge in a full-scale plant (sonix) was found energetically feasible as the benefit obtained as a result of revenue earned from increased biogas production (35–55%) overcompensated the cost spent on sonication [104], making this method suitable for a wide range of feedstock. Despite this, the use of electric energy in comparison to waste heat utilization for thermal pretreatment could be energetically ineffective and a significant drawback to the sonication technique.

Enzymatic pretreatment has been investigated in many previous studies. The enzymes used during or prior to AD include cellulases, cellobiases, endoglucanase, xylanases, pectinases, laccases, versatile peroxidases, as well as  $\alpha$ -amylases and proteases [65]. Deconstruction of lignin and cellulose by enzymes in different stages of hydrolysis can result in the conversion of oligomers and various monomer sugars [105]. Although many enzymes do exist, the application of these in most cases was not practical, and biogas yield in many instances either remained unaffected or dropped [106]. Enzymes can be applied in different ways at different stages of AD. When used upstream during hydrolysis, the sugars released can be potentially consumed by endogenous microorganisms before being further converted into biogas [65]. For cases like this, the endogenous microorganisms are typically eliminated by sterilization (an extra processing step), which incurs additional cost and affects the overall plant economics [65]. Since enzymes for AD are produced from different forms of fungi (e.g., *Aspergillus* and *Trichoderma* genus) [107], they can be used alternatively, so the process cost for producing enzymes can be avoided. Further, an auxiliary enzyme, such as LPMO (Lytic polysaccharide mono-oxygenases) in combination with fungi can also be used for enhancing cellulose degradation [8]. The cost and selectivity are apparently the major drawbacks concerning enzyme application to pretreatment, which, in the context of optimization of AD, calls for further research in future.

Adding certain metals, such as Ca<sup>2+</sup>, Fe<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, and W<sup>6+</sup> [108,109] at specific concentrations, enhances the biogas yield from AD. The enzymes required for essential

bacterial growth are chemically linked with metals (such as those stated above). By supplementing appropriate amounts of these metals, the appropriate levels of nutrients in the anaerobic digester can be maintained, resulting in enhanced substrate degradation and ultimately increased biogas yield [110]. For example, the enzyme complex acetyl-CoA decarboxylase/synthase has an essential role in degrading acetate to methane, which can be further accelerated by adding the metal  $\text{Ni}^{2+}$  [111]. More enzymes were found to be linked to micronutrients or metals [112]. Augmenting heavy metals  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$  at a concentration of 2.5 mg/L to a mesophilic reactor ( $37 \pm 1$  °C) operating on potato waste and cattle manure (50:50), the biogas production was improved [113]. Methane yield was also reported to be increased from a lab-scale digester after adding Fe, Zn, Mn, Cu, and Mo [114]. Using trace elements, the conversion of organic solids can be enhanced allowing stable digester operation at a low organic loading rate (OLR) [115]. However, an excess amount of heavy metals can reduce the methanogen's activity, leading to process failure or collapse [113]. Therefore, the right amount of metal supplementation is essential.

### 3.1.4. Codigestion

Codigesting multiple substrates can improve biogas yield during AD at various combinations and fractions. For instance, methane production increase of up to 65% [116] can be achieved by codigestion of cattle manure with organic wastes (C5 molasses) [117,118], seaweed [47], energy crops [116], food wastes [119], agrowastes [120], and fruit and vegetable wastes [121]. A considerable increase in methane yield from codigestion of many other substrates has also been detected and documented in a comprehensive work by Poulsen T. et al. [122].

Generally, substrate in the AD process is degraded to biomethane by the synergistic interaction of a bacterial consortium, consisting of three functional group of microorganisms involved into the processing steps: hydrolysis, acidogenesis, and methanogenesis. Due to the insufficient buffering capacity, the products of each of these steps can ultimately cause inhibition of methanogenesis. For example, the reduction in pH due to the accumulation of VFAs from acidogenesis caused by the insufficient buffering capacity can result in substantial drop in production of biomethane and thereby loss in AD efficiency.

By deploying codigestion, the digester can be designed for operating at an optimum C:N ratio [123] under which enhanced nutrient balance with improved pH buffering can be achieved. Theoretically, with a C:N ratio of about 25–30:1 [11,124], methanogens use nitrogen to fulfil their protein requirements and optimize the production of methane. At higher ratios, the nitrogen availability depletes quicker, resulting in incomplete methanogenesis, leading to reduced biogas productivity. Conversely, lower C:N ratios give excess nitrogen causing ammonia formation together with an increase in pH and consequently drop in methane yield [125].

Besides C:N ratio enhancement, codigestion can also improve overall macronutrients balance by adjusting the proportion of carbon, nitrogen, phosphorous and sulphur content. The requirement of macronutrients in AD is usually not so high, with a typical ratio of C:N:P:S as 600:15:5:1 [126]. Nonetheless, this depends on the characteristics of feedstock and an individual AD process. Gil et al. [127] showed that substrates with simultaneous high concentrations of N and P, resulting in a combined C:(N + P) ratio between 53 and 40, have a pronounced effect in an increased methane content in the produced biogas. Many other combinations and proportions of macronutrient contents can also lead to possible conditions for enhanced biogas production for a codigestion process, and research into this area is still ongoing.

## 3.2. Reactor

### 3.2.1. Configuration

The way feedstock is converted in AD is greatly dependent on the way the reactor is configured. Generally, two broad classes of substrates, i.e., wet substrate (TS < 15%) and high-solid substrate

(15% < TS < 40%), are treated [128] in a wide range of reactor designs, including anaerobic baffled stacking reactor (ABSR) [129], anaerobic contact process (ACP) [130], anaerobic filter (AF) [131], anaerobic fluidized bed reactor (AFBR) [132], agitated granular sludge bed reactor (AGSBR) [133], anaerobic hybrid reactor (AH) [131], anaerobic membrane reactor (AnMBR) [134], anaerobic sequencing batch reactor (ASBR) [135], anaerobic migrating blanket reactor (AMBR) [136], batch system anaerobic reactor (BSAR) [137], carrier-induced granular sludge bed reactor (CIGSBR) [138], continuously stirred anaerobic bioreactor (CSABR) [139], continuously stirred tank reactor (CSTR) [47], expanded granular sludge bed reactor (EGSBR) [140], fixed-bed reactors (FBR) [141], plug flow reactor (PFR) [142], submerged anaerobic membrane reactor (SAnMBR) [143], super-high-rate anaerobic bioreactor (SAB) [144], temperature phased anaerobic digestion reactor (TPAD) [145], tubular reactor [146], upflow static media anaerobic reactor (USMAR) [147], upflow anaerobic sludge blanket reactor (UASB) [148], and solid-state digester [149]. Selected operational parameters used in these reactors based on existing literature are summarized in Table 4.

**Table 4.** Selected reactor configurations and their operating parameters.

Reactor Configuration	Application	Operational Parameters (Reactor Size, Feedstock Type, Reactor Temperature, pH, OLR and HRT)	Results	Reference
ABSR	Biohydrogen production	3 L, Sucrose, 37 °C, 5.5 ± 0.2 (adjusted), 10–30 gCOD/L, 8 h	<b>HPR:</b> 10.9 ± 1.5 L/L-d, <b>HY:</b> 1.7 ± 0.2 mol/mol-sucrose	[129]
ACP	Methane production	10 L, Olive mill waste water and urea, 35 °C, 7.5 (adjusted), 2 gCOD/L/d, 15 days	<b>MY:</b> 0.15 L-CH <sub>4</sub> /gCOD removed	[130]
AF	Pre-treatment & process performance	60 L, Domestic sewage, 13 °C, N/G, 234 mgCOD/L, 4 h	<b>MC:</b> 70.7 ± 2.9%; <b>MC</b> in <b>AF</b> was found higher than that of <b>AH</b>	[131]
AFBR	Biohydrogen production and waste water treatment	ca 4 L, Synthetic waste water, 37 °C, 4 (adjusted), 10 g/L, 0.5 to 4 h	Max. <b>HPR:</b> 2.36 L/L-h, Max. <b>HY:</b> 1.16 mol/mol-glucose	[132]
AGSB	Biohydrogen production	ca 0.9 L, Glucose, 40 °C, 6.5, 20 gCOD/L, 4, 2, 1 and 0.5 h	<b>HC:</b> 36–41%, <b>HY:</b> 1.4 to 31.5 mol/mol-glucose	[133]
AH	Pre-treatment & process performance of sewage treatment plant	88 L, Domestic sewage, 13 °C, N/G, 340 mgCOD/L, 4 h	<b>MC:</b> 58.9 ± 3.2% (see also <b>AF</b> , given above)	[131]
AnMBR	Biogas generation	2-phase (7 L & 20 L), Cheese whey, 37 ± 2 °C (both phases), 6.5 at start (acidogenic), Max. 19.78 gCOD/L-d (methanogenic), 1 d & 4 d	<b>MC:</b> Max. 70% (methanogenic); biogas production exceeded 10 times reactor volume increased with OLR	[134]
AMBR	Methane production & waste water treatment	12 L, Sucrose base synthetic wastewater, 35 ± 1 °C, 6.5 (adjusted), 30 g/L/d, 12 h	<b>MPR:</b> 6.5 L/L/d with 62.2% average methane based COD removal efficiency	[136]
ASBR	Biogas generation	N/A, Swine waste, 25 °C, 6.8 to 7.4, 0.9 to 5.5 g/L/d, 2 to 6 days	Biogas production rate: 0.9 to 1.8 L/L/d	[135]
BSAR	Biogas generation	1 L (5 units, equal volume), Pig manure (PM) and grass silage (GS), 35 °C, 6.5 to 8.0, 5 PM:GS (1:1, 1:3, 3:1, 1:0, 0:1), 90 days	Max. <b>MY:</b> 304.2 mL/gVS (at OLR 3:1 for PM:GS) Max. cumulative <b>MY:</b> 8517 L (at OLR 3:1 for PM:GS)	[137]
CIGBR	Biohydrogen production & waste water treatment	ca 1 L, Sucrose base waste water, 35 °C, 3 (adjusted), 2.5 to 5 gCOD/L/h, 4 to 8 h	Max. <b>HPR:</b> ~7.3 L/L-h, Max. <b>HY:</b> 3.03 mol/mol-sucrose	[138]
CSAB	Biohydrogen production	ca 1 L, Sucrose, inoculum heat shock, 40 °C, 6.6 ± 0.2, 30 to 40 gCOD/L/h, 0.5 to 6 h	Max. <b>HPR:</b> 15 L/L-h, Optimal <b>HY:</b> 3.5 mol/mol-sucrose	[139]
CSTR	Methane production	5 L, <b>CM</b> and <i>Laminaria digitata</i> , 35 ± 2 °C & 50 ± 1 °C, 8.0 ± 0.3, 2.5 to 2.9 gVS/L/d, 22 days	<b>MY avg.:</b> ca 225 L/kg VS (meso), ca 170 L/kg VS (thermo)	[118]
EGSB	Biogas generation	1 L, Potato-juice, 37 °C, 8 (adjusted to 4, 5, 6 & 7), 2.5 to 4.2 gCOD/L-d, 6 to 10 days	<b>MY avg.:</b> 385 mLCH <sub>4</sub> /gVS; <b>MPR avg.:</b> 1496 mLCH <sub>4</sub> /L-d	[140]
FBR	Biogas generation and biomass development	86 L, Skim milk, whole milk and oleate (variable feeding in 3 periods), 35 °C, 7 to 7.2 (adjusted), 12 g/L (2.4 - 4.15 g oleate/L skim. milk), 426 days	Max. <b>MPR:</b> 33 and 46 mLCH <sub>4</sub> /gVS-d	[141]
PFR	Biogas generation	ca 5 m <sup>3</sup> (field scale plant), Terrestrial weeds and leafy biomass, 25 to 35 °C, N/D, 50 to 100 kg leafy biomass/day, 35 to 70 days	Average biogas yield: 50 L/kg fresh biomass (at OLR: 50 kg/day); 30 to 45 L/kg fresh biomass (at OLR: 100 kg/day)	[142]
SAnMBRs	Biogas generation & waste water treatment	6 L (3 units), Synthetic low strength waste water, 25 to 30 °C, 7.0 ± 0.5 (adjusted), 1.1 to 1.65 kg COD/m <sup>3</sup> /day, 8 to 12 h	Max. <b>MPR avg.:</b> ca 2.9 L/d (HRT: 8, SRT: infinitive), Max. <b>MY average:</b> 0.29 L/gCOD (HRT: 8, SRT: infinitive), Max. <b>specific MY:</b> 0.068 L/MLVSS/d (HRT: 12, SRT: infinitive)	[143]
TPAD	Biogas generation and performance analysis	30 L (meso) & 20 L (thermo), <b>CM</b> , 38 °C (meso) and 58 °C (thermo), 7.00 to 7.75, 2 to 8 gVS/L/d, 14 days	<b>MY:</b> 0.21 to 0.22 L/gVS fed (thermo); 0.15 L/gVS (meso)	[145]
TR	Biogas generation	18 L (4 units), Fruit and vegetable waste, 35 ± 1 °C, 6.8 to 7.6, 2 to 8 gVS/L/d, 12 to 20 days	Max. <b>biogas production rate:</b> 2.62 L/L/d, Max. <b>biogas yield:</b> 707 L/kgVS fed, Max. <b>MC:</b> 65%	[146]
USMAR	Methane production	85 L (3 equal cylinders), Synthetic waste & dry milk, 35 °C, 4.5 to 7.2, 1 to 12 gCOD/L/d, 0.5 to 2 days	<b>MY:</b> 0.1 to 0.2 L/gVS fed with 5 to 13% increase in <b>MC</b> as a result of OLR at 10 gCOD/L/d	[147]
UASB	Hydrogen and methane production	24 L, Cassava waste water, 37 °C, 5.5 (with control), 10 to 30 kg/m <sup>3</sup> -d (biohydrogen reactor) & 2 to 10 kg/m <sup>3</sup> -d (methane reactor, Short (N/G))	Max. <b>HPR:</b> 0.39 L/L-d (at OLR: 25 kg/m <sup>3</sup> -d), Max. <b>HY:</b> 39.83 L/kgCOD fed (at OLR: 25 kg/m <sup>3</sup> -d), Max. <b>MPR:</b> 0.91 L/L-d (at OLR: 8 kg/m <sup>3</sup> -d), Max. <b>MY:</b> 115.23 L/kgCOD (at OLR: 8 kg/m <sup>3</sup> -d)	[148]

**CM:** Cattle manure, **HC:** Hydrogen content, **HPR:** Hydrogen production rate, **HY:** Hydrogen yield, **MC:** methane content, **MLVSS:** Mixed liquid volatile suspended solids, **MPR:** methane production rate, **N/A:** Not accessed, **N/D:** Not determined, **N/G:** Not given.

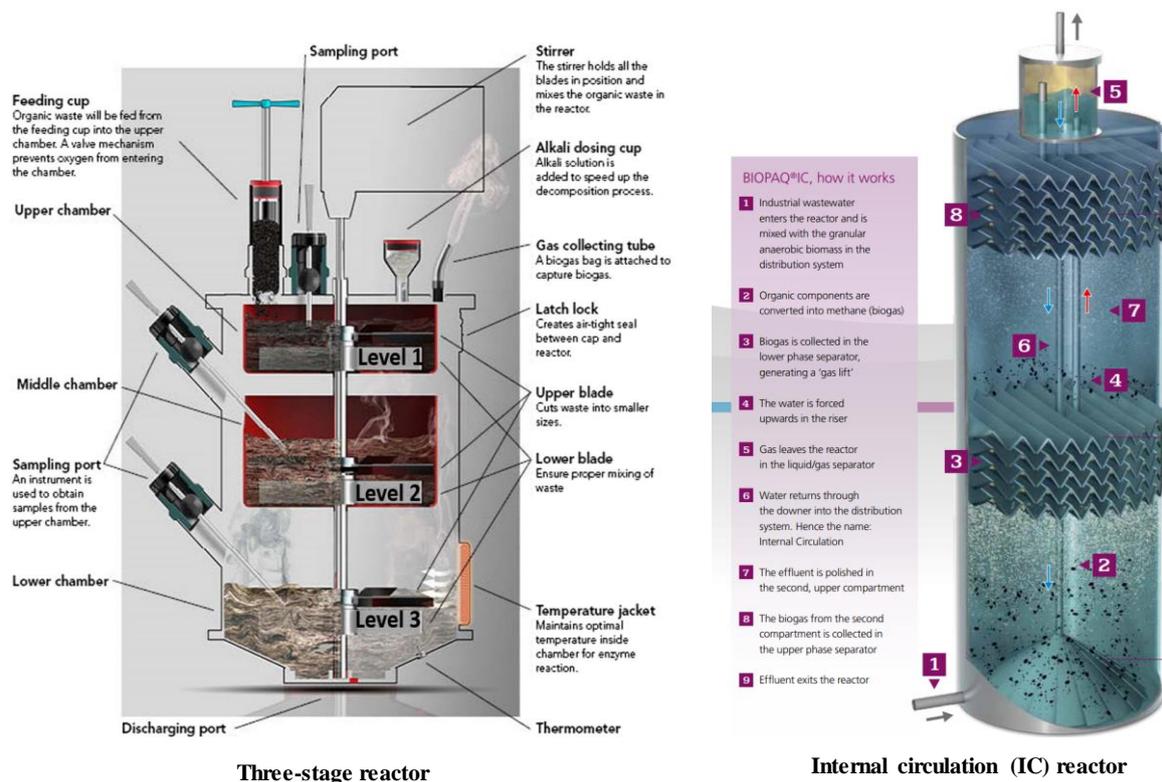
In recent years, the process efficiency and feedstock characteristics (i.e., dry matter content) and reactor systems have been modified to meet the specific criteria of product output, and further development is expected. Broadly, digesters can either be batch or continuous. Batch configurations are straightforward, require less moving parts and are inexpensive [17], but they are generally employed to determine methane potential of substrates where the reactors are subject to the anaerobic environment for a long period, until a degree of digestion close to the theoretical maximum is achieved. However, in a real life scenario, constant gas production is more desirable, requiring continuous digestion.

Continuous digestion system can be designed with single or multiple digesters. In a single stage digestion system, all steps in microbial degradation, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, occur simultaneously in a single reactor vessel. For the majority of anaerobic digestion applications, single stage reactors are still dominant because of their simplicity in design and cost [150]. So far, an array of substrates such as food waste [151], vegetable waste [152], sludge [151], municipal solid waste (MSW), biowaste [150], and livestock manure [153] have been treated by this configuration and process performance is optimized in a vast number of cases. For example, optimum methane recovery using a single stage reactor (CSTR, PFR, UASB, ASBR, and TPAD) for livestock manure (i.e., cattle manure, pig manure, and poultry manure) was shown to be achieved and even maximized by recirculating the process liquid back into the reactor [154]. By digestate recirculation, liquid retention time increases and the microbe wash out from the process consequently decreases [155].

In a two-stage system, the first step involves feeding material into the first digestion tank (acidogenic reactor), where hydrolysis, acetogenesis, and acidogenesis take place. The partially digested materials from this reactor are then introduced into the second stage (methanogenic reactor), where the methane is produced. Compared to the single stage digestion, the two-stage process allows fast and efficient formation of biogas in the second stage where methane recovery of volatile solids can exceed 90% in exceptional cases [156,157]. However, due to the complexity in design, two-stage reactors have a higher cost [150].

The three-stage reactor concept was developed in the early 1990s [158]. In this configuration, the first phase involves semi-anaerobic hydrolysis of feedstock at low hydraulic retention time and the removal of un-degraded materials to the secondary reactor for acidogenesis. From the secondary reactor, output liquids and solids are transferred to a tertiary reactor where methane-rich biogas is produced. Likewise, in a two-stage system, a part, or all of the effluent from the tertiary reactor can be recirculated back to the digester depending on the targeted HRT and process efficiency [159]. According to the study by Kim et al. [159], using a three-stage reactor, COD removal efficiency of over 95% was achieved for food waste AD. In a more recent study [7], an 11–23% increase in methane yield for a three-stage codigestion of food waste and horse manure was reported.

In addition to the configurations mention above, AD systems have undergone several modifications over the last few decades to address the retention efficiency of slow-growing methanogens. With the development of the UASB reactor, where a dense sludge bed allows methanogenic sludge to maintain within the reactor [160], the improvement in retention efficiency was found to be significant. So far, numerous UASB reactor types are being used for treating waste and wastewater effluents of various characteristics. Among these, the internal circulation (IC) reactor was observed to exhibit high efficiencies in terms of considerable feasibility, robust resistance to outside accidents, high organic loading rate, and low investment cost [161]. Membrane bioreactors (MBR) are another technique quite successfully able to retain active biomass in the system. By employing a membrane, both cells and inhibitory components within the bioreactor can be separated and as such, an efficient biological process can be achieved [162]. Available membrane reactors and their application in the context of AD can be learned from a comprehensive review authored by Visvanathan and Abeynayaka [162]. Figure 4 illustrates two examples of novel reactor configurations that are used in both lab- and industrial-scale applications.



**Figure 4.** Example of two novel anaerobic reactor types—reproduced with permission from the original authors [7,161].

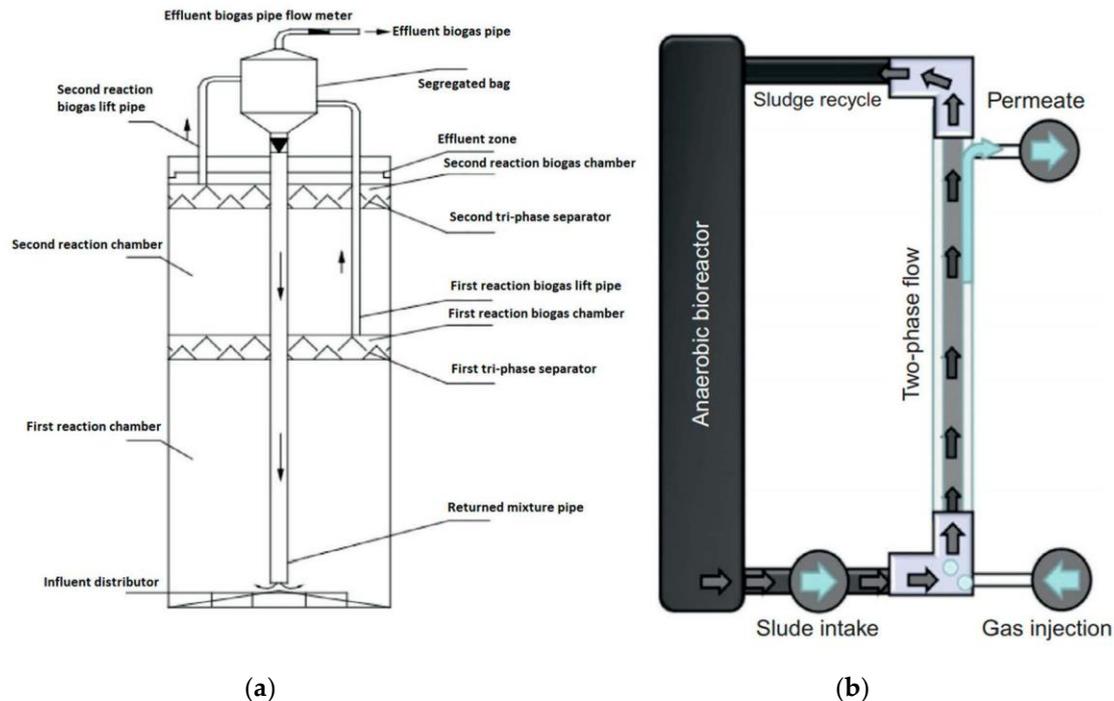
Apart from bioreactor design, the developments in biosensor technologies and molecular biology techniques can enable understanding complex microbiological processes and potential disturbances much earlier than the conventional AD processes that do not involve any sensors for that matter. Reviews of the current progress in sensor technologies for controlling AD is documented excellently by Gaida et al. [163].

### 3.2.2. Mixing

Mixing, to a great extent, can determine the performance and cost of an AD system. Mixing promotes contact between microorganisms, substrates, and nutrients and provides uniform temperature distribution in the digester. Adequate mixing can reduce sedimentation and foaming caused by floating fat with adhering gas bubbles or by filamentous microorganisms, such as *Microthrix* or *Nocardia* [164]. Reactors equipped with mixing tend to produce more biogas [165] than those without, although contrasting results do exist [166,167]. Mixing can be accomplished by means of mechanical (employing a mixer), hydraulic (liquid recirculation), and pneumatic (recirculation of gases) techniques [168] at various frequencies (continuously or intermittently with several hours or several times in an hour during a day) [11] and intensities (gentle, intermittent, and rigorous rotation speed). Ong et al. [169] showed that employing intermittent mixing, mass transfer from the liquid phase to the gas phase greatly enhanced resulting in an increased gas release as much as 70% higher than the periods without mixing. In addition, among various mixing intensities, gentle mixing leads to the formation of aggregates and prevents methane-producing organisms from being washed out by the liquid and thereby proved to be more effective [170].

Despite the benefits of increased gas release, mixing does have an extra cost and requires an energy input. Continuous mixing in a full-scale AD can demand as much as ~50% of the total plant energy [171], with mixer motor start-up consuming 2.5 % additional energy [172]. Therefore, mixing should be compromised or chosen carefully, given the feedstock type and AD technology. Strategies

like switching from continuous to intermittent mixing mode could save over 25% of the energy demand and ensure higher plant productivity [173]. Additionally, using innovative technologies such as a gas-lifting reactor (that works on biogas's rising tendency for partial recirculation and digester's agitation) can provide the effect of mixing without requiring a mixer, and has been shown to result in improved plant economies [174]. Two innovative mixing configurations operating with the airlifting mixing concept are shown in Figure 5.



**Figure 5.** Two novel configurations (a,b) of airlifting bioreactor agitating sludge without having an installed mixer—reproduced with permission from the original authors [175,176]. (a) biogas-lift reactor (BLR): right upward arrow—first biogas lift included with sludge and water; middle downward arrow—flow of sludge and water; left upward arrow—second biogas lift with separation of sludge and water. (b) gas-lift membrane bioreactor: the flow directions of materials are indicated with the arrows and texts in the body of the figure.

### 3.3. Temperature

The AD temperature depends on the tolerance of the microorganisms and is categorized as psychrophilic, mesophilic, thermophilic, or extremophilic [177] established at temperature ranges of 4–25 °C, 30–40 °C, 50–60 °C, and >65 °C, respectively [177,178]. Although anaerobic digestion can be achieved with temperatures lower than 20 °C [179], below 10 °C, the degradation is three times slower than the normal mesophilic process with methanogenesis becoming the rate-limiting step [180]. With higher temperatures, such as those between mesophilic and thermophilic regimes, anaerobic digestion tends to have a perfect environment for biological degradation resulting in high rates of hydrolysis and consequently high biogas yields. The correlation between the reaction rate and the biological process with temperature is typically represented by Equation (8) below [181]:

$$k_T = k_{20} \theta^{(T-20)} \quad (8)$$

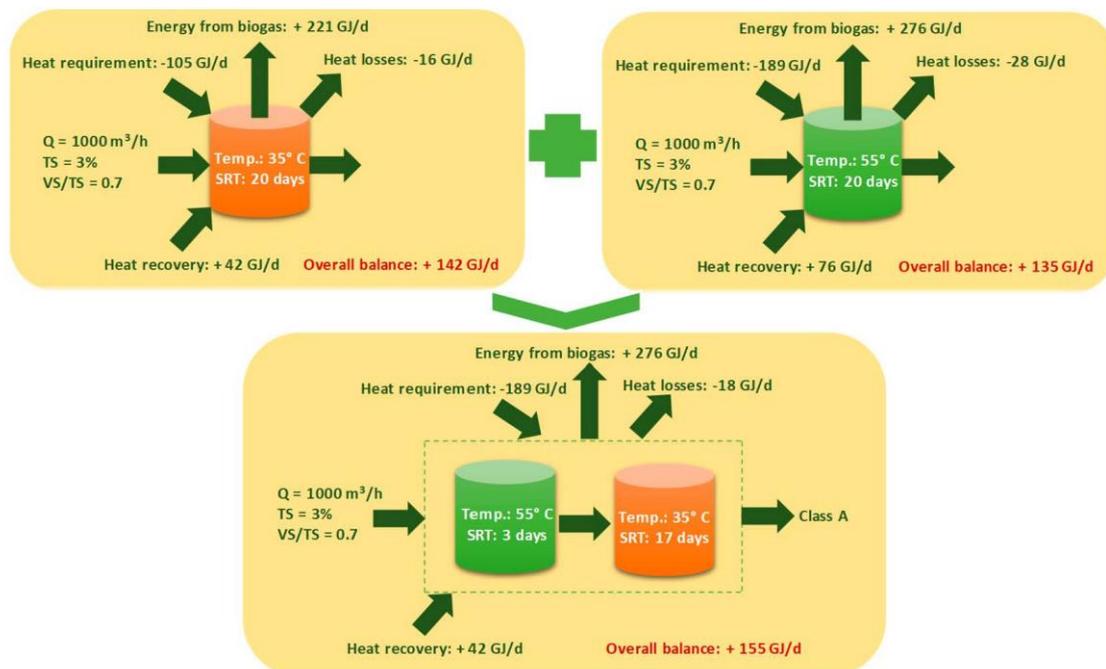
where  $k_T$  denotes the reaction rate constant at temperature  $T$ ,  $k_{20}$  represents the reaction rate constant at 20 °C,  $\theta$  is the temperature activity constant, and  $T$  denotes the temperature.

The methane production amount in thermophilic AD is almost identical to mesophilic AD, but higher temperatures improve the production rate [182], and reduces the requirement of high operational

HRT and consequently reducing the reactor size [183]. However, thermophilic digestion system can be energy intensive, unstable, and sensitive to inhibition [184] (see Section 3.5), which, in addition to the robustness of the operation, is why mesophilic processes are presently the most preferred techniques implemented at industrial scale AD [185].

For a given operational temperature type, the fluctuation of a few degrees of temperature can have a severe impact on methane yield, as microorganisms adjust to one certain temperature and re-adaptation corresponding to a different temperature requires an alternated microbial structure. Noticeably, a variation in mesophilic temperature of  $\pm 4$  °C and thermophilic temperature of  $\pm 1$  °C was found to result in a sharp decrease in biogas yield [19,186].

Within physio-microbial activities, some AD processing phases are more influenced by the temperature than others. For example, the temperature requirements for optimizing the growth of methanogens bacteria, specifically the mesophilic methanogens species, may differ from the temperature requirements for optimizing hydrolysis or acidification [19]. Investigation of phase-specific temperature effects has been conducted and it was found that the temperature-phased (70 and 55 °C in the two successive stages) digesters, separating hydrolysis and methanogenesis, produced better results in terms of biogas yield and organic matter removal than the thermophilic or mesophilic digestion alone [187]. Similar results were demonstrated in previous research works by the authors Puchajda et al. [188,189]. Moreover, a staged digester combining thermophilic (55 °C) and extremophilic (68 °C) temperatures was reported to accelerate hydrolysis of recalcitrant organics, resulting in higher biogas yields compared to a thermophilic or extremophilic reactor alone [190]. Figure 6 illustrates the energy generation comparison between a single and staged digester operated at different temperature conditions.



**Figure 6.** Higher energy generation from a two-stage thermophilic-mesophilic digestion compared to a single stage mesophilic or thermophilic digestion alone—modified from Puchajda et al. [189].

Temperature optimization by employing various technological options such as reactor design [187], OLR or HRT variation [191], pretreatment [192], and post-treatment [190] have been the major topics investigated in the existing literature. Moreover, incorporating computer simulation with the use of advanced modelling tools, such as ADM1 (Anaerobic Digestion Model No. 1), is gaining popularity in recent research [193].

### 3.4. pH

The pH is measured to indicate the health of anaerobic microorganisms and the performance of AD system [17]. A variation of pH in the magnitude of 0.5 can result in a substantial change in microbial metabolism influencing reaction kinetics and produced gas yield. Although feasible anaerobic digestion can be achieved within the pH of 5.5 and 8.5 [194], methanogens are highly sensitive to the pH change and generally optimize at a pH of close to 7 [177]. A pH under 6.3 or over 7.8 can adversely affect methanogenesis with a tendency of process failure to occur [195]. However, unlike methanogenesis, the other processing steps (e.g., hydrolysis and acidogenesis), can potentially optimize between pH of 5.5 and 6.5 [196]. With regard to the intermediates, specifically the VFAs produced during the acidogenesis if not metabolized into products, the pH can be significantly lowered (<3), eventually leading to process collapse. Process disturbances can also result due to high alkalinity caused by the formation of total ammonium nitrogen (TAN) during hydrolysis. Generally, the pH range of extreme acidic (~3 or less) or extreme alkaline (~12 or higher) can be inhibitory to the acidogenesis [197] and limit the rate of hydrolysis.

The development of various chemical species during biochemical interaction (e.g.,  $\text{NH}_3$ ,  $\text{CO}_3^{2-}$ , and  $\text{CH}_3\text{COO}^-$ ) can cause a considerable impact in digestate pH variation. For example, the formation of ammonium carbonate ( $(\text{NH}_4)_2\text{CO}_3$ ) or reduction of  $\text{CO}_2$  from the digestate liquid can directly lead to a pH increase [198,199]. Also, the production of basic cations (e.g.,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ) or the reduction of multivalent anions (e.g.,  $\text{SO}_4^{2-}$ ,  $\text{Fe}(\text{OH})_3$ ) can cause electric charge imbalances in the digestate liquid resulting in a pH rise. Similar to observations in pH increases, the precipitation of carbonates (e.g., calcite  $\text{CaCO}_3$ ) or the fatty acids as a consequence of high organic loading could be the critical factors influencing pH reduction. Kovács et al. [200] showed that the protein-rich substrates (i.e., meat extracts), tend to wane buffering capacity at an elevated organic loading input resulting in pH drop. The tendency of the various ions originating from various degradation pathways to influence pH is shown by Figure 7.

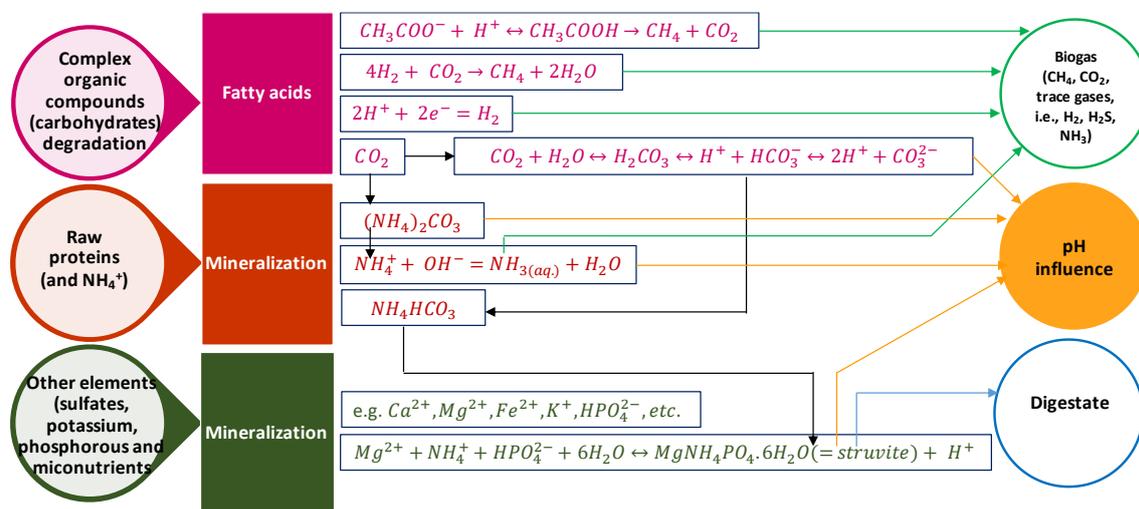


Figure 7. Interaction of degradation pathways and the formation of products influencing pH.

To control pH fluctuation and to operate AD process at the desired pH range, modern biogas plants are often installed with an automatic pH controller. The primary objective of the controller is to help balancing the system buffer (alkalinity) by adding an appropriate neutralizing agent at an appropriate concentration when necessary. Strong bases (e.g.,  $\text{NaOH}$ ) or carbonate salts (e.g.,  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$ ) and acids (e.g.,  $\text{HCl}$ ) are primarily the components used as chemicals to achieve a pH increase or decrease. Moreover, indirect pH control can be applied by manipulating operating parameters including a reduction in organic loading rate [201], digestate recirculation [202], HRT variation [203] and codigestion [204].

The controlling of pH, whether achieved by the direct addition of a buffer or by the manipulation of the operating conditions, requires a careful and sophisticated application to avoid disturbances to methanogenesis and further to biogas yield. Robust controlling of pH by incorporating multiple parameters towards an optimum methane yield using sensor technologies is a growing field of research in AD.

### 3.5. HRT

Retention time is an important parameter used for design and optimization of anaerobic digestion. Retention time refers to both hydraulic retention time (HRT) and solid retention time (SRT). HRT represents the retention time of the liquid phase, whereas SRT denotes the retention of the microbial culture in the digester. In an anaerobic reactor system where the feedstock and microbial mixed cultures are present at the same phase, the HRT equals SRT. AD of food waste, kitchen waste, and municipal solid waste are the examples for which HRT is essentially SRT and vice-versa, while for the substrates like waste activated sludge and primary sludge, the interaction between solids and microbial cultures are biphasic making HRT and SRT different. HRT and SRT are typically represented by Equations (9) and (10) [205]:

$$\text{HRT} = \frac{V}{Q} \quad (9)$$

$$\text{SRT} = \frac{V \cdot X}{Q_x \cdot X_x} \quad (10)$$

where, V: Individual reactor volume [m<sup>3</sup>]; Q: Influent flow rate [m<sup>3</sup>/d]; X: Mixed liquid suspended solids in an individual reactor [mg/L]; Q<sub>x</sub>: Excess biosolids removal rate [m<sup>3</sup>/d]; X<sub>x</sub>: Mixed liquid suspended solids in excess biosolids flow [mg/L].

In general, the choice of HRT differs based on feedstock composition, reactor volume, processes, and temperature. Substrates rich in starch and sugar can be easily digested [177], and as such, hydrolysis is less or not required, resulting in shorter retention times, while longer retention times are required for fiber and cellulose plant matter as hydrolysis of these substrates is slow and rate limiting. High reactor temperature increases the decomposition rate and consequently lowers HRT, which is why most thermophilic reactors are generally operated at a lower HRT than mesophilic reactors. Comparing two HRT types, shorter HRT risks bacterial mobilization including the build-up of higher molecular weight VFAs and consequently elevated stress to the methanogens [206]. Conversely, a longer HRT increases the digester size [183]. Therefore, the optimal operational HRT is usually neither too long nor too short and in the majority of cases lies between 10 and 25 days, although a very high HRT in the order of 50–100 or more days may be needed for digesters operated in colder climates [206].

With regard to the different phases of AD, typically a longer HRT is preferred for methanogenesis to match the slower growth rate of methanogens compared to acidogens. This also validates the fact that many current digesters are designed in a multistaged manner so that acidogenesis and methanogenesis are technically separated into two different volumes allowing each group of microorganisms to operate at optimal conditions [207]. The correlation between the different bacterial species and different levels of HRT has been studied and depicted in a recent paper by Yang et al. [208].

In the case of anaerobic digestion of sludge type feedstock, consideration of both HRT and SRT are essential. For a given HRT, longer SRT usually provides sufficient time for methanogens to complete biological activities and result in a high biogas yield. With an operating HRT of 12 h, infinitive SRT for a submerged anaerobic membrane bioreactor (SAnMBR) was found to double the methane production rate (L/L/d) than that for SRT of 30 days [143]. However, contrasting results were also reported [209], where the shorter SRT (or increased OLR) was found to give a higher biogas production rate (L/L/d) [209]. This was explained by the fact that a raised OLR may support an increased methanogenic population and hence enhanced microbial activities leading to higher biogas productivity, but the higher VFA accumulation might indicate that the process is already overloaded. Therefore, the balance between the choice of SRT and OLR is critical for an optimum AD. At the start-up

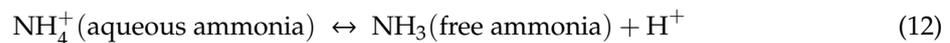
period, in general, a long acclimatization time under longer SRT and lower OLR are required for the satisfactory development of slow-growing populations [210], while in the stable period, a relatively shorter operational SRT may be needed allowing improved process performance including a higher methane yield. In a past study, shorter SRT was deliberately used to control the production of VFA by shifting the microbial communities from one route of production to another, for example, from acetogenotrophic to hydrogenotrophic [211]. A similar effect (but with a shift from methane to VFA production), was observed while the HRT was shortened [212].

### 3.6. Ammonia

Ammonia is produced from the nitrogen-containing matters in the feedstock (i.e., from the degradation of proteins and urea in the manure). In the aqueous phase, it is present in two forms, ammonium ions ( $\text{NH}_4^+$ ) and free ammonia (FA) or unionized ammonia ( $\text{NH}_3$ ). Together, this is called the total ammonia nitrogen (TAN) [213]. In anaerobic digestion, only a partial fraction of the organic nitrogen (Kjeldahl nitrogen) is biologically degraded to inorganic ammonia with the proportion spanning between 34 and 80% [214–216]. Most of the ammonia is usually generated during hydrolysis [217], and the type produced is influenced by the factors such as a change in temperature, pH, inoculum or microbial community. The FA and ionic ammonia with relation to TAN are typically expressed by Equation (11) below [218,219]:

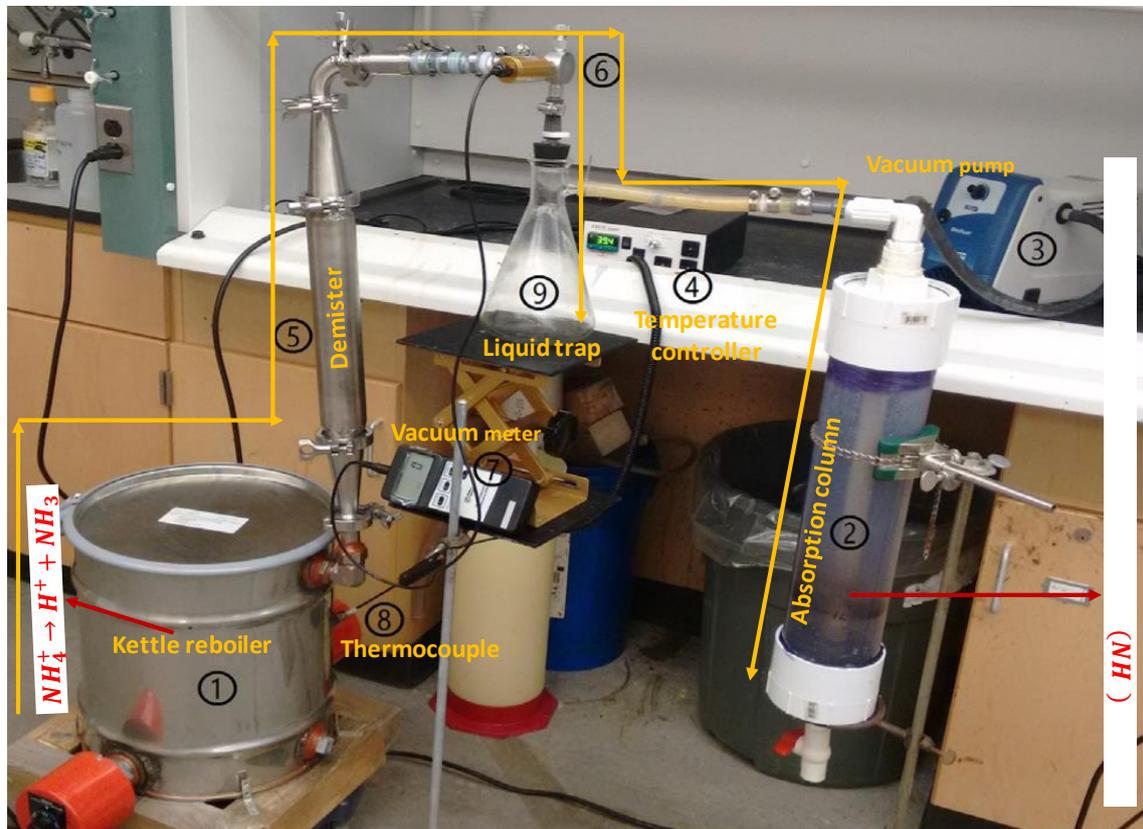
$$\text{NH}_3\text{-N} = \frac{\text{NH}_4^+\text{-N}}{1 + \frac{10^{-\text{pH}}}{10^{-(0.09018 + \frac{2729.92}{T})}}} \quad (11)$$

FA is a strong candidate for methanogen inhibition. It is membrane permeable, and when it diffuses through the cells, proton imbalance or variation in intracellular pH occurs causing inhibition of enzymatic reactions [220]. High temperature combined with a high pH decreases the solubility of ammonia resulting in the dissociation equilibrium of aqueous ammonia to shift towards free ammonia leading to the production of this ammonia to be dominated over ionic ammonia (see Equations (11) and (12)).



At mesophilic temperature with a pH of 7, approximately 1.25% of TAN converts to FA, while for the same temperature and a pH of 8, approximately 11.25% TAN transforms to FA; suggesting FA to be ten times more toxic to methanogens at a pH of 8 than at a pH of 7 [221]. Generally, a FA concentration between 1.7 and 14 gN/L is inhibitory to the methanogens, mainly to the acetoclastic species [219], which results in 50% or more reduction in methane yield [222], and in the worst case, the process will collapse. The degree at which methanogens are affected by the level of ammonia differs across several bacteria types and environmental conditions. An investigation by Niu et al. [223] observed that acetoclastic methanogens are more sensitive than hydrogenotrophic methanogens, while other researchers [224] observed the opposite, where hydrogenotrophic methanogens were found to be less tolerant. Some species of both acetoclastic and hydrogenotrophic methanogens were observed to tolerate TAN at levels higher than 10 g/L [225]. At elevated ammonia levels, for example, with a concentration greater than 3 g/L, the shift of acetoclastic methanogens to syntrophic acetate oxidation was observed that consequently led to a two-fold decrease in methane yield [226]. With regard to environmental conditions, thermophilic methanogens are generally deemed to tolerate higher levels of free ammonia than the mesophilic methanogens [227], but the process was found to be unstable and easily prone to inhibition [228]. Therefore, to obtain an optimum AD, a careful choice of process condition, particularly operating temperature, pH, type of inoculum, and feedstock, are necessary, so the level of free ammonia, as per recommendation from the published literature, is kept below 0.2 g/L [229]. Lowering FA and overcoming ammonia toxicity is achieved by employing various physical and chemical strategies including air stripping [230], use of zeolite [231], membrane filtration [232], diluting

substrates [229], post-treatment of AD effluent [233], bioaugmentation [234], anaerobic ammonia oxidation [235], ultrasonication [235], microwave irradiation [236], struvite precipitation [237], and C/N ratio adjustment [238], all of which are comprehensively reviewed in a recent paper authored by Krakat et al. [213]. A novel ammonia recovery system using vacuum thermal stripping, an acid absorption process developed by the authors Ukwuani and Tao [239], is shown in Figure 8.



**Figure 8.** Innovative vacuum thermal stripping–acid absorption process for ammonia recovery—reproduced with permission from the original authors [239].

### 3.7. VFA

VFAs, mainly composed of acetic acid/acetate, propionic acid/propionate, butyric acid/butyrate, valeric acid/valerate, caproic acid/caproate, and enanthic acid/enanthate [240], are the essential intermediates during the anaerobic digestion process. The majority of the VFAs degrade to acetate and further to methane via methanogenesis through acetoclastic and hydrogenotrophic pathways (see Figure 2). In an AD process, a higher VFA conversion efficiency is usually desirable, as this allows the achievement of greater stability in methane production and balance in different production stages.

During the lifetime of an AD plant, it can suffer from nonoptimal VFA formation because of various operational disturbances caused by the feedstock organic loading rate, pH, temperature, and  $\text{H}_2$  partial pressure change. Accumulated VFAs, depending on the levels and types, can be toxic and in extreme cases inhibitory to the process. For example, the development of propionic acid, in excess of levels as low as 1 g/L, can be extremely detrimental to methanogens, resulting in reduced or stagnated production of methane [241]. Other VFAs, such as butyrate and acetate, although not as potent as propionic acid, can cause process collapse when the concentration reaches to 10 g/L or more [241]. Therefore, for a well-controlled AD system, formation of a high concentration of VFAs of any type is not recommended, as this leads to decreases in the potential production of methane and consequently the diminished AD efficiency. Several measures have been proposed to enable accelerated conversion of VFAs into methane and discussed in the previously cited research articles. Among these, the selection

of an appropriate reactor type, operating temperature, pH, hydrogen partial pressure, organic loading rate, and chemical additives are widely considered to be essential [242].

The selection of an appropriate reactor design can play a pivotal role in determining VFA conversion. For example, multistage reactors over single stage reactors can allow establishing operating conditions simultaneously suitable for optimal interaction of different groups of bacteria in individual AD phases, resulting in a balance in exchange of products and reactants, including increased conversion of VFAs. Despite this, due to the design complexity and high capital cost, the two-stage reactors are not preferred in many real applications and, alternatively, the design optimization of a single stage reactor towards maximizing VFA reduction have been given special attention. Installing baffles at various levels of a granular bed reactor [243] and employing membrane bioreactors [244] are some of the examples identified to have promising results in accelerated degradation of VFAs. Nevertheless, the future research on either reducing the cost of a multistage reactor or incorporating more advanced features to single stage reactors on optimal utilization of VFAs for improving AD efficiency is desirable.

Temperature is another variable having a substantial impact on VFA production and utilization. Since the presence and growth rate of microbial species are highly influenced by the level of temperature changes, their intercommunication that leads to the production and conversion of VFAs also changes. The effect of various temperature ranges (psychrophilic & mesophilic; 4–20 °C & 20–50 °C, respectively) on both production and composition of VFAs during hydrolysis have been studied, and it has been observed that with the rise in temperature, the rate of hydrolysis increases, resulting in increased solubility of carbohydrates and proteins, and consequently an accelerated production of VFAs. However, this temperature change did not have any noticeable impact on the VFA composition change with only some reductions in acetate production from 55–43% corresponding to the temperature rise between 4 and 14 °C [245]. Another study [246] investigated the change in temperature on the conversion of different VFA components. Here, the degree of conversion on specific VFA components (i.e., acetate, propionate and butyrate), as a result of sudden temperature change (from 63–55 °C) was examined, and the results suggested that the sudden increase in temperature influenced methanogenic populations resulting in unconverted (partial) propionate. However, as soon as the reactors were exposed back to the original temperature (55 °C), microorganisms adapted faster, giving increased methane yield followed by an improved propionate conversion extent. Some past researchers also studied temperature changes in combination with feedstock pretreatment on conversion of VFAs [247]. Their results, although inconsistent, reported that the extent of VFA conversion is a function of both temperature, feedstock and the given pretreatment technique, as one or all of these parameters directly influences the population and the type of microbial culture, dictating the way at which VFAs are produced and utilized towards biogas production. Identifying the correlation between microbial growth patterns and their mechanism towards VFA production and degradation will, therefore, be an interesting future research topic in this direction.

In addition to temperature, the pH in an anaerobic reactor can also be an indication of the status of VFAs. Varying pH over the range of extreme acidic (less than 3.0) and extreme alkaline (above 12.0) on accumulated VFAs was examined, and it was found that the VFA buildup reached their peak once the pH went to 10. A similar phenomenon was observed by another study where the SFAs (short-chain fatty acids) were found peaking when pH ranged between 8.0–10.0 compared to that of 3.0–7.0 [248]. This was thought to be caused by the fact that at an alkaline pH, there was higher availability of soluble proteins and carbohydrates than those at a neutral or acidic pH. In contrast, a study dealing with chicken manure with a high TS as a substrate found the VFA formation to be an inverse function of pH change. Here, the authors [216] performed further analysis on accumulated VFA types, and observed that the unutilized VFAs are mainly composed of unionized VFA types, called U-VFAs as calculated based on the total VFA concentration using Equation (13) below:

$$U - VFA = VFA \left( 10^{(pK_a - p^H)} / (1 + 10^{(pK_a - p^H)}) \right) \quad (13)$$

where the dissociation constant values of acids in water ( $K_a$ ) at 353 °C were taken from Weast (1987).

These VFAs, unlike ionized VFAs, are characteristically more toxic to methanogenesis and strongly linked with pH variation. The past study by [216] observed that U-VFA concentration as low as 10 mg/L in acetic acid was already a cause of inhibition to methanogenesis. However, the research on U-VFA and its role on various anaerobic digestion aspects are scarce, and its impact on the overall AD optimization and effectivity have no solid proof yet.

The partial pressure of  $H_2$  has a direct impact on the interaction of several groups of bacteria that exchange VFAs. For example, the syntrophic acetate oxidation, symbiotically linked to the conversion of acetate to methane, is thermodynamically hindered (when Gibbs free energy of this reaction goes towards positive) if the partial pressure of  $H_2$  exceeds the acceptable operating limit (i.e., 74 Pa [249]). Also, the propionate degradation can be severely reduced as a result of  $H_2$  partial pressure rise [250], and, in the worst case, the process can stall when Gibbs free energy for this conversion becomes more positive [251]. The relationship between  $H_2$  partial pressure and the interaction between  $H_2$ -producing and  $H_2$ -consuming anaerobes towards influencing individual VFA component are complex and are not clearly understood. Nonetheless, the research on  $H_2$  partial pressure manipulation by employing various external techniques is ongoing [249], and some promising results in this line are already achieved.

As with the factors above, the organic loading rate (OLR) is another parameter strongly correlated with the VFA yield and its conversion. Independent of the feedstock type, a high OLR in general leads to VFA accumulation as a result of an imbalance between the growth rates of methanogenic archaea and VFA producing bacteria and speed of methane and VFA producing bacteria. Also, a high OLR can contribute to changes in VFA composition from low molecular weight ones to the high molecular weight ones, for example, from acetic acid to n-butyric acid [244], which eventually can promote methane inhibition. To achieve improved VFA utilization, a sophisticated OLR adjustment with reduced or withdrawn feeding were proved to be beneficial in previous research [118,244,252]. However, the range of optimal OLR values corresponding to the reactor and feedstock type, microbial adaptation pattern, and methane yield remain unexplored.

### 3.8. OLR

The amount of raw-material added to an anaerobic digester per day per unit volume is called the organic loading rate, and is typically expressed as Equation (14):

$$OLR = \frac{C}{HRT} \quad (14)$$

where C is the feed concentration in g-VS/L, and HRT is the hydraulic retention time.

OLR is an essential controlling parameter in AD, and its deliberate variation can determine the degree of digestion for a broad level of influent input. In a typical anaerobic digester application, a high operating OLR is usually preferred, as this allows enriched bacterial species, reduced reactor sizes, lowered heating requirement and a reduced investment cost [253]. Several reactor configurations have been investigated to achieve a high OLR, wherein the primary aim was to reduce HRT either by adding water to the substrate or to recirculate digestate back to the main reactor [254]. By lowering HRT of a lab-scale spiral automatic circulation (SPAC) bioreactor, a high OLR equaling to 300 kg/m<sup>3</sup>/d [144] was successfully accomplished. Another reactor type, up-flow anaerobic fixed bed (UAFB), was also reported to provide high operational OLR, but the maximum OLR level for these configurations was reported to be much lower than the SPAC type. However, a lowered HRT may lead to microbial washout and eventually digester failure. Also, there are possibilities of VFA accumulation [255] as the anaerobic digester operates either with higher OLR or forced to run with a lower HRT. Generally, methanogenic reactors were found to have higher stability at a somewhat higher HRT, typically between 10 and 25 days for CSTR type configurations. Nevertheless, Zhang et al. [254] recently used a novel feeding strategy where OLR was kept constant while HRT was simultaneously reduced. This

results in a reduction in ammonia nitrogen inhibition with concomitant improvement in methane yield. Various other approaches of OLR optimization leading to enhanced biogas production are continually being researched. Among others, microbial management [256] and use of additives [90] are gaining constant popularity. Table 5 lists a range of OLR applied in recent AD publications (during the year 2018) on a number of feedstocks including agricultural residue [257], algae [258], energy crops [259], industrial wastes [260,261], fish waste [262], food waste [263–266], horticulture waste [267], manure [268], MSW [269], and sludge [270,271].

**Table 5.** A range of OLR variations used in selected recent publications on anaerobic digestion.

OLR	CH <sub>4</sub> Yield	Aim	Feedstock	Reference
0.4 to 3.1 kg COD/m <sup>3</sup>	Maximum (0.46 LCH <sub>4</sub> /gCOD <sub>removed</sub> ) at OLR of 2.5 kg COD/m <sup>3</sup>	Co-digestion	Rice straw and pig manure	[257]
1 to 4 gVS/L/d for methane reactor; 3 to 12 gVS/L/d for H <sub>2</sub> reactor	Methane production maximized at OLR of 2 gVS/L/d and thereafter decreased. H <sub>2</sub> production maximized at OLR of 6 gVS/L/d	Co-production of H <sub>2</sub> and CH <sub>4</sub>	Macro-algae <i>Laminaria digitata</i> and micro-algae <i>Arthrospira plantensis</i>	[258]
30, 60 and 90 gVS/L	Methane yield for all the co-digestion types maximized at OLR of 30 gvs/l and 60 gVS/L	Co-digestion performance	Sweet potato vine and animal manure	[259]
2.5 to 27.7 gVS/L	Ammonia inhibition at OLR > 20 gVS/L	Ammonia inhibition	Tannery fleshing, municipal solid waste, chrome shaving and others	[260]
0.4 to 0.7 gCOD/L/d	Methane yield decreased with increased OLR	Pilot scale two stage AD	Slaughter house waste	[261]
1.5 to 4.3 g/L/d	Maximum methane yield at OLR of 3.5 g/L/d	Methane production by ammonium tolerant microorganisms	Protein rich fish silage	[262]
1, 2 & 3 gVS/L/d	70% and 73% reduction of SMY and SCOD for OLR increment from 1 to 3 gVS/L/d	Semi-continuous AD at different psychrophilic range	Food waste	[263]
Various	Specific gas production (0.88 m <sup>3</sup> biogas/kg <sub>vs</sub> ) at two stage reactor was found higher than that of (0.75 m <sup>3</sup> biogas/kg <sub>vs</sub> ) single stage reactor for an optimum OLR of 3.5 kg <sub>vs</sub> /m <sup>3</sup> /d	Comparison between single and two stage reactor performance	Food waste	[264]
2.4 and 3.6 gCOD/d	Higher OLR led to reactor's acidification problem and hence affected methane yield	Performance and metagenomics analyses of single and two stage thermophilic anaerobic digestion	Cheese wastes	[265]
4.6 and 8.6 kgCOD/m <sup>3</sup> /d	The maximum methane productivity peaked to 2.78 L/L/d at OLR of 8.6 kgCOD/m <sup>3</sup> /d, but the system was unstable	Effect of feeding with or without dilution	Food waste	[266]
2.0 to 6.0 gVS/L/d	Methane yield decreased as OLR increased for both two-stage and co-digestion reactors	Comparison between two-stage and co-digestion AD	Food waste and horticulture waste	[267]
1.53 to 5.04 gVS/L	0.44 LCH <sub>4</sub> /gVS at OLR of 5.04 gVS/L	Determination of kinetics constant	Co-digestion of cattle manure and municipal food waste	[268]
Reactor ASBR: 0.93–25.0 gCOD/L.d Reactor AMBR: 1.04–19.65 gCOD/L.d	Maximum biogas yield at OLR of 10.08 gCOD/L.d, Biogas production decreased for OLR > 18.52 gCOD/L.d	Effect of OLR and series reactor AD	Composting leachate	[269]
0.5, 1.5 and 2.0 VS/L <sub>sludge</sub> /d	H <sub>2</sub> uptake by homoacetogens increased at higher OLR resulting acetate accumulation	Acetate concentration during in situ methane upgradation	Sludge and H <sub>2</sub> ; fluromethane as inhibitor	[270]
1.12 to 3.88 kgCOD/m <sup>3</sup> /d	Methane yield continued to increase up to OLR of 2 kgCOD/m <sup>3</sup> /d. Methane production inhibited at OLR > 3.8 kgCOD/m <sup>3</sup> /d	Co-digestion	Beverage waste and sewage sludge	[271]

### 3.9. Pressure

The effect of pressure on anaerobic digestion has not been studied extensively. Although anaerobic digestion typically occurs at atmospheric pressure, due to the accumulation and exchange of different

gases into the reactor headspace, over or under pressure on a liquid surface can be developed. Based on the previous findings, it was reported that lower pressures on liquid surfaces resulted in higher biogas yields, as CH<sub>4</sub> solubility increases with pressure [272]. In an earlier study [273], the pressure change effect of various gas species was investigated using reaction models. The results showed that the partial pressure increase of CO<sub>2</sub> led to increasing the digester pH level, which consequently lowered the nonionized hydrogen sulfide concentration, reducing gas toxicity. Additionally, the hydrostatic pressure level within the digester can also affect the production of methane. Past experiments [274] observed that the methanogenesis activities maximized at digester depth of 4–5 m (400–500 mm H<sub>2</sub>O) for a digester of over 10 m height. By reducing the height of this digester (i.e., making it horizontally oriented), a reduced hydrostatic pressure can be established, resulting in improved methanogenesis.

Anaerobic reactors working with high pressures (i.e., pressurized reactors), have been developed and investigated in lab-scale studies. As high pressure increases CO<sub>2</sub> solubility, the biogas released from the anaerobic digester partially strips of CO<sub>2</sub>, giving net rise in CH<sub>4</sub> concentration. By using a high pressure within an anaerobic digester, a methane level of above 95% was achieved [275]. However, in some cases, elevated pressures in an anaerobic reactor did not produce a satisfactory improvement in biogas production. Technical challenges associated with leakages in the reactor system, pH decrement, and investment costs are identified as a few of the drawbacks of the pressurized reactor concept.

#### 4. Conclusions

Optimizing operational parameters towards increasing methane yield has been and will be the most adopted technique within the application of the anaerobic digestion process in both industrial and lab-based studies. The role of individual and multiple parameters combined in different aspects of anaerobic digestion has been explored in the present review and the following stand out points, as conclusions, are expressed:

- (1) Feedstock physical and chemical compositions substantially affect biogas production. Among the various types of feedstock materials, animal manure is still the dominant substrate or cosubstrate for biogas production because of its operational advantages of pH buffering and C:N ratio optimization. Lignin-rich substrates are found to be recalcitrant, while lipids are expected to have a high potential to boost methane. Nevertheless, LCFA inhibition from lignin remains to be of concern. Low or no lignin feedstock such as algae is an interesting biogas yield promoter. However, LCFA inhibition, increased pH level, and ammonia inhibition are highlighted as some of the barriers. For counteracting feedstock induced operational problems, codigestion, pretreatment, and use of additives are utilized in current R&D and real-life applications.
- (2) Enhancing feedstock accessibility allows accelerated biological degradation and consequently high AD efficiency. Pretreatment and codigestion are broadly used options promoting feedstock accessibility. However, the choice of the pretreatment method is feedstock-dependent and often is a compromise between cost and energy. For pretreatment of lignocellulosic biomass (e.g., animal manure), by overcoming the recalcitrant lignin or crystalline cellulose barrier, the biogas production can be enhanced where approaches like steam explosion, enzyme addition, and sonication at present are widespread. For substrates with high-fat content, saponification is preferably used, while for algae-like substrates, thermal pretreatment is considered an option. Despite this, almost all the conventional pretreatment methods have both success and failure, as some pretreatment options are easily amenable, while others have side-effects that counteract their positive effects. Within the novel pretreatment approaches, a combination of various pretreatment methods has been examined, and the obtained results are reported to be promising. Additionally, as for future studies, exploiting genomic sequencing as a means of understanding feedstock degradation prior to anaerobic digestion is suggested.
- (3) Manipulating reactor designs for achieving an optimum AD process performance has been emphasized, revealing many innovative approaches currently in practice. In terms of continuous operation, staged reactors give substantial increases in methane yields due to the establishment

of appropriate microbiological conditions at different anaerobic digestion phases. Consequently, three-staged reactor configurations have been developed and reported to be an attractive option in optimizing methane production. Moreover, an anaerobic membrane reactor, internal circulation reactor, and super-high-rate reactor are some of the novel configurations shown to facilitate efficient high solid substrate treatment by increasing bacterial cells containment and separating simultaneous removal of gases, solids, and liquids. Therefore, these reactor approaches are becoming increasingly popular.

- (4) Among the various operational temperature regimes (psychrophilic, mesophilic, and thermophilic), the choice of an appropriate regime is largely investment and geographic specific. Thermophilic temperature enhances methane conversion rate and controls pathogens in the digestate liquids. However, the high heat requirement makes its application expensive. The recent investigations towards an optimum temperature system suggest that multitemperature, staged-digesters offer suitable conditions for diverse microbial activities, and hence give high biogas production efficiencies. By employing high-temperature post-treatment of digestate, further improvement in methane production has already been demonstrated.
- (5) The pH affects the degree of conversion at different AD steps and the quality of the residual digestate. Optimal methanogenesis and biogas production occurs at around pH 7. A host of factors, such as ammonium formation, bicarbonate decomposition, mineralization and reduction of multivalent ions, and struvite formation result in digestate pH fluctuations. To enable pH controlling to the desired value, adding acid or basic solutions are traditionally the major options. Moreover, online pH monitoring and alert systems are also implemented in modern day applications.
- (6) HRT is directly linked to the size of the anaerobic reactor, and a low HRT usually allows investment reduction. Among the all bacteria and archaea, methanogens grow slowly, and for these microorganisms, a higher HRT is required. Both feed rate and feed type influence the HRT. Consequently, the feedstock OLR regulation is a usual approach for HRT optimization. SRT, another term intertwined with HRT, represents the microbial culture and biomass retention in an operating digester. SRT is often optimized by incorporating OLR variation. A recent achievement suggests that a deliberate SRT variation can result in a shift in bacterial culture, causing a change in reaction pattern from one group to the other.
- (7) Ammonia is produced via the degradation of proteins and nitrogen in the feedstock. Among the two forms of ammonia (free ammonia and ionized ammonia), free ammonia is very toxic to methanogens and is a strong function of combined pH and temperature. Lowering the generation of ammonia during AD has been targeted in a variety of approaches (see Section 3.6), among which, using ammonia tolerant microorganisms (or bio-augmentation) or combined thermal stripping and absorption process are some of the novel techniques receiving constant attention.
- (8) VFAs are the intermediate products required for conversion to methane. Some of the VFA components are more sensitive than the others to methanogens, e.g., propionate. Unutilized VFAs accumulate, and in the worst case halt the production of biogas. To achieve increased VFA utilization and as such improved methane yield, regulation of AD process parameters such as temperature, OLR, pH, and H<sub>2</sub> partial pressure is critical. Moreover, many additives and trace metals of various origins have been suggested to have an improved utilization of VFAs.
- (9) OLR refers to the amount of feedstock treated by a reactor on a daily basis. OLR variation allows the optimization of HRT, pH, VFA, ammonia, and methane production. High operational OLR enables reducing the size of the reactor and accordingly the investment cost. However, as a result of high OLR, implications such as bacteria wash out, VFA accumulation, or methane yield reduction can be experienced. In recent applications, OLR control has been used to suppress ammonia inhibition. Furthermore, a novel three-stage reactor configuration has been shown to successfully achieve high OLR treatment without compromising the production of biogas.

- (10) Anaerobic digestion operates typically at atmospheric pressure, but recent investigations have identified that high-pressure systems are also possible. High pressure allows the increase of dissolved CO<sub>2</sub> in the liquid phase and consequently increased methane composition in the biogas. The other aspects of anaerobic digester pressure, such as partial pressure of headspace gas components and variation of hydrostatic pressure levels, were mentioned as potential causes of fluctuation in the production of methane.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3417/9/9/1915/s1>, Figure S1: Artwork Figures Revised, Table S1: Table Artwork Tables revised.

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