energies

Review

Granular Sludge Bed Processes in Anaerobic Digestion of Particle-Rich Substrates

Fasil Ayelegn Tassew [1,*], Wenche Hennie Bergland [1], Carlos Dinamarca [1], Roald Kommedal [2] and Rune Bakke [1]

[1] Department of Process, Energy and Environmental Technology, University of South-Eastern Norway, Kjølnes ring 56, 3918 Porsgrunn, Norway
[2] Institute of Chemistry, Biosciences and Environmental Engineering Stavanger, University of Stavanger, 4021 Stavanger, Norway
* Correspondence: fasil.a.tassew@usn.no

Received: 8 July 2019; Accepted: 28 July 2019; Published: 31 July 2019

Abstract: Granular sludge bed (GSB) anaerobic digestion (AD) is a well-established method for efficient wastewater treatment, limited, however, by the wastewater particle content. This review is carried out to investigate how and to what extent feed particles influence GSB to evaluate the applicability of GSB to various types of slurries that are abundantly available. Sludge bed microorganisms evidently have mechanisms to retain feed particles for digestion. Disintegration and hydrolysis of such particulates are often the rate-limiting steps in AD. GSB running on particle-rich substrates and factors that affect these processes are stdied especially. Disintegration and hydrolysis models are therefore reviewed. How particles may influence other key processes within GSB is also discussed. Based on this, limitations and strategies for effective digestion of particle-rich substrates in high-rate AD reactors are evaluated.

Keywords: high-rate anaerobic digestion; granular sludge; disintegration; hydrolysis; suspended solids; particulates

1. Introduction

Anaerobic digestion (AD) has been used to treat organic wastes for renewable energy production for decades. Due to the ongoing shift towards renewable energy, biomethane produced by AD is getting increased attention as an energy carrier [1] and as a potential chemical platform for synthesis of added value products such as polysaccharides, single-cell protein, and polyhydroxyalkanoates [2]. Biomethane can be produced from a wide variety of organic feedstocks such as agricultural and domestic wastes [3]. However, the low energy density of some of the largest feed sources, such as sludge and manure, limits production rates and process efficiency in continuous flow stirred tank reactors (CSTR) currently used for sludge and manure AD [4]. Such processes without efficient biomass retention are voluminous and therefore expensive to build and operate [5]. High-rate AD, such as up-flow anaerobic sludge bed (UASB) reactors, are used to obtain more sustainable energy recovery as it provides high COD (Chemical Oxygen Demand) removal even at high OLR (Organic Loading Rate) and short HRT (Hydraulic Retention Time). Its design is simple and compact, requiring relatively low construction cost. It has, however, some limitations regarding feed composition that require discussion, especially: The particle content of some of the most abundant substrates, such as sludge and manure slurries, is well above the levels considered appropriate as UASB reactor influent [6]. Large quantities of slurries that can and should be used for biogas production exist (e.g., the Norwegian government aims to utilize 30% of manure slurries for AD by 2020 while <1% was used according to a 2011 report [5]), and this study can contribute to expanding the applicability of high-rate sludge bed AD. We address some of the main challenges associated with high-rate anaerobic digestion of particle-rich substrates with special emphasis on manure as a substrate, due to its abundance. Most high-rate AD processes in operation depend on granular sludge to retain sufficient active biomass. Granules are formed by the aggregation of microorganisms that develop into dense masses with sedimentation velocity high enough to avoid washout even under high hydraulic load [7]. It is observed that UASB reactors treating particle-rich manure slurries also accumulates suspended solids from the feed, forming an additional suspended fraction together with the granules [8,9]. The influence of such solids on AD is not understood well, leading some experts to claim that granular sludge bed (GSB) processes may not be appropriate for particle-rich substrates. [10]. It appears, however, that a significant fraction of feed particles can be digested and enhance methane production [11]. This review was undertaken to investigate to what extent and how sludge bed high-rate AD can be used to treat particle-rich substrates. We aim to find more evidence for particle digestion in granular sludge beds, identify process limitations, and find appropriate kinetic models in order to establish design criteria for such processes. There is little directly relevant literature on the topic, limiting this review to mainly indirectly relevant literature. The review covers particle-rich substrates characteristics; particle disintegration and hydrolysis, including models for such; physical characteristics of granular sludge; sludge bed reactor designs and observations of particle effects.

2. Anaerobic Sludge Bed Processes

Various anaerobic processes have been used for the treatment of wastewater for decades. These processes include septic and Imhoff tanks, which are some of the earliest methods used to treat wastewater. They are simple systems where low to moderate COD and suspended solids removal can be achieved. Anaerobic lagoons are also used for larger volumes of wastewater or manure. In this system, the wastewater is held for a prolonged time. The lagoon must have sufficient depth to ensure anaerobic condition. It is a low maintenance process but it is inefficient and has a negative environmental impact due to gas release and odor. With the increasing understanding of the underlying anaerobic processes, newer, more controlled reactors were developed. In continuous flow stirred tank reactors complete mixing of reactor contents is assumed, and both design and operation are simple. Horizontal plug flow and anaerobic sequential batch reactors have also been used over the years. These reactors are generally low-rate systems with maintenance requirements but require long HRT and large reactor volumes. The need for fast, efficient, and more environmentally friendly alternatives for anaerobic wastewater treatment led to the development of sludge bed processes. High loading rate, short HRT, and efficient conversion of organic compounds to biogas were possible due to increased bioreactor densities of active biomass by decoupling sludge retention time (SRT) from HRT. Starting from the 1970s, a number of high-rate reactors have been developed. Some of the most common high-rate reactors and their advantages and disadvantages are summarized in Table 1. Lettinga [12] specified four essential requirements that enabled the proliferation of anaerobic sludge bed processes. The first is the formation of balanced and immobilized microorganisms. In many anaerobic reactors, immobilization is achieved in the form of microbial aggregates or granules. The second requirement is high settleability of microbial aggregates in order to ensure that the microbial biomass remains in the reactor even if high flow velocity is applied (SRT > HRT). Sludge bed reactors are often equipped with gas–solid or gas–liquid–solid separators that aid retaining granular sludge. Third, a high degree of contact between sludge and substrate must be achieved (convective mass transfer). The last requirement is the presence of a high rate of mass transfer in and out of the microbial aggregates (mainly diffusive mass transfer).

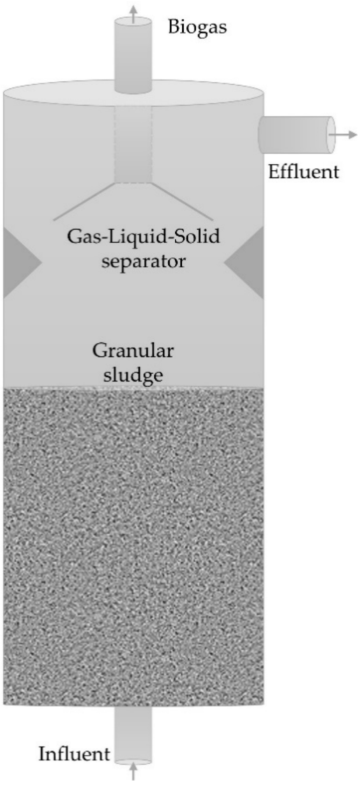
Table 1. Applications, advantages, and disadvantages of commonly used high-rate reactors.

Name	Common Applications	Advantages	Disadvantages
Anaerobic contact process (ACP)	Wastewater containing suspended solids	Good contact between biomass and substrate Efficiency	Poor sludge settling Complex system
Anaerobic filter (AF)	Low or high strength wastewater	Requires small area Stable sludge Long service time	Difficulty to maintain contact between sludge and wastewater Affected by accumulation of non-degradable matter Difficult clean-up process
Up-flow anaerobic sludge blanket (UASB)	High strength industrial wastewater	Simple design Relatively low cost Low excess sludge production High removal efficiency	Recovery time may be long after stress conditions May require expert maintenance Internal mixing may not be optimal (dead zones)
Anaerobic fluidized bed (AFBR)	Industrial wastewater (dilute, low in suspended solids)	High biomass retention High surface area due to attached growth of microbes on carrier media	Difficult scale-up Expensive
Expanded granular sludge bed (EGSB)	Low strength wastewater (low suspended solids)	Improved mixing (no dead zones) High removal efficiency for soluble constituents	Suspended solids removal is low
Internal circulation (IC)	Low or high strength wastewater	High organic loading High contact between sludge and wastewater Larger granular sludge	Sludge washout may be a problem Low granular sludge strength
Anaerobic baffled reactor (ABR)	Low or high strength wastewater	No mechanical mixing Biomass do not need good settling properties Tolerates shock loads	Influent distribution is not even through reactor Variable sludge retention

Up-Flow Anaerobic Sludge Blanket (UASB)

UASB is a high-rate AD reactor usually used for the treatment of industrial wastewater, invented by Lettinga et al. [13] in the 1970s. After a slow start, there has been a rapid growth in its application over the last decades. There has also been an increase in design variations where the two most common are: EGSB (Expanded Granular Sludge Blanket) which is a reactor that is essentially a UASB with higher up-flow rate and recirculation of effluent; IC (Internal Circulation) reactor where two UASB-type reactors are stacked and used in series and enables efficient mixing without external recycle pumping. These encompass one or more up-flow anaerobic sludge blankets, so this review does not distinguish between such concepts and considers all as UASB, in accordance with the view of Prof. Lettinga (personal communication). UASB reactors differ from conventional AD by the facts that they can handle much higher organic loading rates [13,14] (up to 15–40 gCOD L^{-3} d^{-1}) and short hydraulic retention time (0.3–7 days). The reason for the high efficiency of UASB and other high-rate reactors is that the sludge retention time (SRT) is decoupled from the hydraulic retention time (HRT) so that SRT > HRT while SRT = HRT in conventional CSTR reactors. Typical UASB SRT values are in excess of 30 d and biomass concentration can reach up to 100 kg/m^3 at the bottom of the sludge bed [10]. This is achieved when the microorganisms are aggregated in granules that have higher densities than the wastewater/substrate they are treating, such that the granular sludge is retained in the reactor even if high feed flow rates are used. The anaerobic microorganisms naturally aggregate into dense granules of 0.1–8 mm diameter under UASB conditions [15]. Size and density of the granules are important characteristics because they influence the settling of granules and mass transfer between the granules and the surrounding liquid. The inlet is at the bottom and the outlet is at the top of UASB, as illustrated in Figure 1.

An up-flow velocity of 0.7–1.0 m/h is recommended by Tilley et al. (2014) [16] so that granules remain in the reactor, however, up-flow velocities of 1–3 m/h are also typically used with mean settling velocities ranging from 20 to 100 m/h [7]. Fragments of granules and particles introduced in the feed may, however, be susceptible to wash-out from the reactor due to lower settling velocities. UASB reactors are equipped with gas–liquid–solid separators that are located at the top (Figure 1) to primarily separate liquid and biogas for collection. Such arrangements are also intended to help retain particles carried out of the sludge bed by gas bubbles that can be knocked off when passing through the separator. The separator narrows so that the particles can settle back down. UASB is suitable to treat high strength industrial wastewater such as from pulp and paper processing, tanneries, distilleries, chemical, and pharmaceuticals industries while substrates with high-suspended solids, high lipid, and protein content are considered less appropriate [17]. For example, wastewater from slaughterhouses is considered unsuitable for treatment in UASB because it contains high concentrations of lipids and suspended solids [17]. Accumulation of lipids and suspended solids in the sludge bed supposedly leads to biomass wash-out and process failure. Difficulties experienced with the treatment of high particulate substrates, such as manure, that contains straws and other long fibers are mainly mechanical as it often leads to pipe blockage and channeling. During storage, however, such fibers tend to float if the substrate is left undisturbed for a day or more [18,19] and a supernatant can be withdrawn and used as UASB substrate. Even at suspended solids concentrations above what is considered appropriate for UASB, high conversion rates and yields, and substantial conversion of the particulates at low HRT is achieved [11]. The question remains: How can this be, given the slow disintegration and hydrolysis of particulates and the low settling velocity of such particles?



(a)

(b)

(c)

Figure 1. Up-flow anaerobic sludge bed (UASB) reactor components (**a**) and examples of granular sludge (**b**,**c**).

3. Particle-Rich Substrates

Several sources of substrates are used for the production of biogas by anaerobic digestion. The most common sources include industrial waste, food waste, agricultural waste, and manure. Each substrate has their own physical and chemical characteristics that make them suitable or unsuitable for a given AD reactor. For example, UASB reactor is considered ideal for the treatment of industrial and municipal wastewater with low total solids (TS) and particulate content while CSTR reactors are instead used for pumpable particle-rich substrates, such as manure slurries, AD processes can be classified into three categories based on the total solids (TS) content of the substrates used. These are: Wet (0–10% TS), semi-dry (10–20% TS), and dry (above 20% TS) [20]. Increase in TS content up to around 30% can increase the biogas production [21], while above this level biogas production may be curbed by mass transfer limitation: The substrate is simply too thick to allow efficient mixing and mass transfer of metabolites, resulting in low methane yield [22]. Batstone and Jensen evaluated appropriate reactors depending on solid content as summarized in Figure 2. Later cases are also added to this figure, such as Bergland et al. [11] who demonstrated in lab-scale that particle-rich substrates (pig manure slurry supernatant) can be efficiently treated in high-rate AD. There are potential benefits of using particle-rich substrates (degradable organics) since it implies relatively high digestible substrate content per total liquid volume and therefore high substrate energy density. This can imply increased biogas production efficiency, reduced cost of feed transport, compact reactors, and low operational energy demand [20]. Massé et al. [23] carried out high rate digestion of dairy manure with TS of 35% using dry anaerobic digestion (PDAD) at 20 °C in sequential batch reactor and 21 d cycle length. They achieved an average methane yield of 152 ± 8 L CH_4/kgVS and VS removal of 42 ± 4% (UASB is certainly not suitable for such high TS). Such sludge bed high-rate reactors are often used for substrates with low suspended solids content, usually <1% TS but there are studies that show sludge bed treatment of relatively high-solids containing substrates: Fujihira et al. [24] used a modified 'anaerobic baffled reactor' (ABR) system at HRT of 7.3 d and OLR of 4.8 gCOD L^{-3} d^{-1} to treat a substrate that contains high levels of suspended solids (7 ± 12 gTSS/L) and showed that a COD removal of 95% was achievable. Andalib et al. [25] showed, using corn stillage (by-product from bioethanol production) substrate with 47 gTSS/L, that 78% TSS removal was achievable by using anaerobic fluidized bed reactor (AFBR) at HRT of 3.5 d (with a methane yield of 0.345 L CH_4/g COD consumed). Successful treatment of substrates with TS content above 10% at short HRT has also been reported in more conventional UASB-type reactors: Fang et al. [26] reported 90% COD removal capability for both UASB and EGSB in treating palm oil mill Effluent (POME) with substrate TS well over 10% at HRT of 5 d. A study carried out by Borja et al. [27] even showed that UASB reactors are capable of treating POME at HRT of <1 d with suspended solids concentrations reaching 5.4 g/L and OLR reaching up to 17.3 gCOD L^{-3} d^{-1} at HRT of 0.9 d.

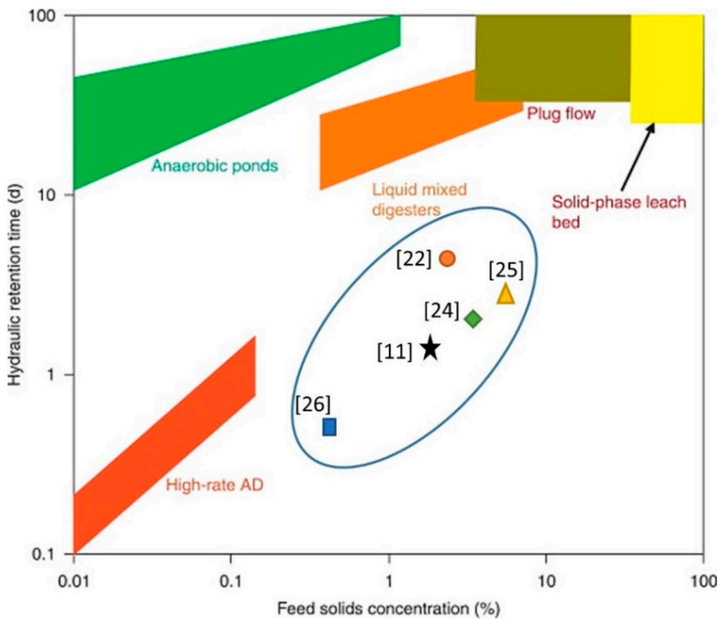


Figure 2. Hydraulic retention time for various anaerobic digestion (AD) reactors depending on feed solids content (Adapted from Batstone and Jensen [28]) where data from [11,23,25–27] are added.

3.1. Manure

Physical and chemical properties of manure influence how it can be used as a substrate for anaerobic digesters. Manure collected from storage facilities usually contains a large amount of water that has been used for cleaning and flushing raw manure from barns, especially for pig and cow manure. In addition, bedding materials, unused animal feed, and other materials can enter the water-manure mix. Animal age, sex, health, weight, type (ruminant or non-ruminant), whether pregnant or not also affects the chemical composition of manure [29]. Manure has high contents of Carbon (C), Nitrogen (N), and Phosphorus (P). The C:N ratio is an important characteristic of manure in AD because it is linked to ammonia inhibition both when too low [30] and too high [31]. Manure with high solids content is dominated by high C content and hence usually has a high C:N ratio whereas liquid manure contains a lower C:N ratio [32]. A comparison of typical total solids content (dry matter) of raw manure with liquid manure is provided in Table 2.

Table 2. Comparison of typical dry matter content of different types of manure with and without dilution.

Manure Type	Dry Matter (%)	
	Raw Manure (As Excreted)	**Liquid Manure**
Pig	9–11 [29,32]	2–5 [33]
Cattle	8–12 [29,32]	3–8 [33]
Poultry	25–35 [31,34]	<15 [34]
Horse	14–20 [29,35]	<15 [35]
Sheep	25 [29]	-

Livestock farming usually incorporates manure storage pits, in which its physical and chemical properties are altered. During storage, denser contents settle at the bottom, a liquid fraction with less large particles (manure supernatant) establish above and lighter material, such as straw, float at the top. Hence characteristics, including density and organic content, differ with time and height from which the manure is taken in storage pits. Anaerobic conditions during manure storage can lead to emission of a significant amount of biogas and further alter the chemical composition of manure [36,37], largely dependent on storage temperature [19]. Feng et al. [37] estimated methane loss of 1–46% for pig manure and 1–2% for cattle manure. Manure also undergoes hydrolysis, fermentation, and acidogenesis while stored, potentially leading to improved digestibility [19].

3.2. Swine Manure Characteristics

Swine manure is abundant and AD of swine sludge is extensively studied. Typically about 4–5 kg manure per day per animal is produced (corresponding to organic content of 0.4–0.5 kgCOD/d with C:N ratio of 7–8) [38]. Reported values for total and volatile solids per animal are usually 0.5–0.8 and 0.4–0.5 kg d^{-1}, respectively [29]. Swine manure has a high content of solid particulates that are difficult to digest [24,39]. Straw or saw-dust (as bedding material), other fibers, and lignocellulosic particulates pose a challenge in achieving the full biogas potential. Møller et al. [40] showed through batch experiments that the average methane potential from swine manure (pig and sow) is only about 60% of the manure COD value (calculated based on the volume of methane per mass of volatile solids). The relatively high content of protein and lipids further challenge high-rate AD due to potential ammonia inhibition and foam formation. Møller et al. [40] estimated the protein and lipid content of swine manure and found average values of 240 and 143 g/kgVS, respectively [40]. Ammonia comes from deamination of urine and amino acids and is split between free ammonia (NH_3) and ammonium ion (NH_4^+) depending on pH and temperature, both of which can play an inhibitory role in the Methanogenesis step [30,41]. In addition, the degradation of amino acids can lead to the formation of hydrogen sulphide (H_2S) which exacerbates inhibition [41,42]. One of the strategies used to alleviate ammonia inhibition as well as increase biogas generation is co-digestion of swine manure with other substrates that are low on N-based compounds [43,44]. Common substrates for co-digestion with swine manure are crop residue, food waste, and municipal organic waste. Reports on the topic have focused on co-digestion of manure in UASB or UASB-type reactors [45–47]. Bergland et al. [11] and Nordgård et al. [48] have, however, shown the feasibility of single substrate swine manure supernatant digestion in high-rate reactors, explained by UASB population adaptation to much higher ammonia concentrations than those causing inhibition in conventional manure AD.

4. Disintegration of Solid Particulates

Total solids are comprised of soluble and particulate contents (Figure 3). The particulate fraction requires disintegration as well as hydrolysis steps before it can be taken up by microorganisms. Disintegration is defined as the (slow) release from a complex composite material of macromolecules that will be further hydrolyzed. Disintegration or hydrolysis is assumed to be the rate-limiting step in AD when particle-rich substrates are applied [49]. Inactive or dead biomass is considered as part of the particulate fraction available for digestion. The disintegration of particulates results in degradable and non-degradable fractions. The non-degradable fractions are soluble and non-soluble inert particulates, whereas the degradable fractions consist of biopolymers, in ADM1 (Anaerobic Digestion Model No. 1) limited to polysaccharides, proteins, and lipids [49–51]. Disintegration is mostly described by first-order kinetics, sometimes assumed to be part of the hydrolysis step. Disintegration in AD reactors depends on various factors such as particle size, morphology, strength, temperature, and chemical composition. Substrates with high solids content are often pretreated before AD to speed up disintegration and hydrolysis. There are thermal, chemical, mechanical, and biological pretreatment methods (and combinations of such), including milling, alkaline treatment, thermal treatment, ultrasound agitation, and composting [52]. An objective of the pretreatment is to increase the surface area of the solid particulates available for enzymatic activity [53].

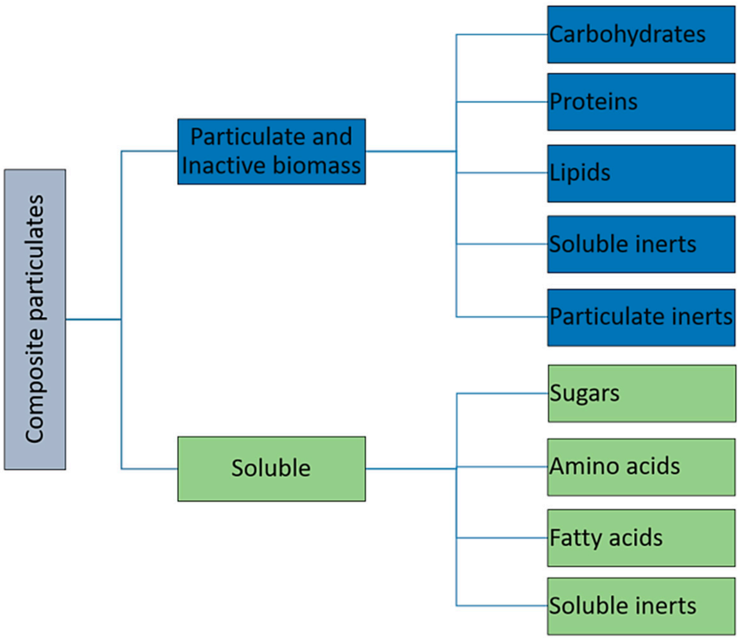


Figure 3. Composite particulate fractions according to the Anaerobic Digestion Model No. 1 (ADM1) model. Particulate contents and dead biomass undergo disintegration and hydrolysis to become soluble.

4.1. Factors Affecting the Degradation of Particles

Due to the typical short HRT of UASB, feed particles must somehow be retained longer than HRT to be degraded but mechanisms for such are not described in the literature so indirect evidence is considered. UASB reactors treating particle-rich manure slurries accumulate suspended solids from the feed, forming an additional suspended fraction together with the granules [8,9] and a significant fraction of feed particles can be digested and contribute significantly to methane production at low HRT [11]. The density of such feed particles can be low so that they remain suspended during storage and in feed containers [11], implying that their sedimentation characteristics are such that they should have reactor retention time similar to HRT unless somehow 'captured' by the sludge bed. Such capture mechanisms can be by adsorption to granular sludge and/or granular sludge (or fragments of such) colonizing the feed particles. Fletcher (1994) [54] claims that "molecular biology has demonstrated that bacteria are able to "sense" surface environments, altering their pattern of gene expression" and have a diversity of attachment mechanisms. Several studies have demonstrated the importance of extracellular polymeric substance (EPS) in granular sludge (such as summarized by van Lier et al. (2017) [15]) with similar composition and roles as in biofilms so it seems likely that the bacteria on the surface of granules can use the mechanisms described by Fletcher (1994) [54] to actively attach particles for the purpose of retaining and digesting.

Mahmoud et al. [8] identified three categories of factors that affect solid removal in up-flow reactors. These are: (1) Reactor operational conditions (such as temperature OLR, HRT, and up-flow velocity), (2) influent characteristics, and (3) sludge bed characteristics. They noted that temperature increase leads to an increase in solids removal. They proposed that an increase in temperature decreases viscosity, leading to reduced hydraulic shear force acting on the particles. Alternatively or additionally, increased temperature will enhance solubility rates (especially of fats and lipids in organic particles) and increase depolymerization. Other studies have also reported an increase in the removal of solids with temperature [55–57]. Increase in HRT also increases the removal of solids, however, HRT is linked with OLR for a given substrate and these are intertwined parameters with organic compound concentrations, and up-flow velocity, so it is not clear that HRT has a direct effect on particle degradation. The results presented by Bergland et al. [11] did not seem to suggest this since a similar particle contribution to the biogas production was observed for HRT from 40 to 2 h. Sludge blankets that entrap suspended solids can enhance digestion, as described above, but may also lead to a decrease in settleablity of the granular sludge. This may interfere with process performance unless it is countered by disintegration and hydrolysis. It is therefore important to understand what the fate of the accumulated suspended solids can be. Accumulated particulates interact with the surrounding liquid phase and microorganisms in the sludge bed. Large particles undergo separation into smaller ones due to a combination of structural weakening due to hydration, hydrodynamic shear force or mixing, enzymatic dissolution, etc. By and large, disintegration depends on particle retention time—hence, the reactor SRT is the controlling process parameter. Continuous and prolonged entrapment of suspended solids that are voluminous and not degraded sufficiently fast can decrease sludge bed particle settleability, and eventually to sudden washout of the sludge bed. Lettinga et al. [6] observed such phenomenon both in lab-scale and in full-scale reactors. Suspended solids may also accumulate at the reactor top, creating an inverted solid profile, leading to inadequate contact between particulates and microbial biomass especially in digesters with gas-mixers [58,59]. This is explained by the formation of foam and produced biogas lifting up low-density particles by floatation.

4.2. Hydrolysis of Particulates

Hydrolysis is defined as a chemical process of decomposition involving the splitting of a molecular covalent bond and the addition of the hydrogen cation and the hydroxide anion of water [60]. Hydrolysis is the second step in anaerobic digestion of organic substances where macromolecules are degraded into smaller molecules by bacterially excreted extracellular enzymes [61]. Strictly speaking, this mechanism is one of several depolymerization reactions possible, but in the terminology of wastewater and sludge treatment, hydrolysis is used for the net sum of these. Stoichiometrically, hydrolysis products are monomers, oligomers, or polymers of reduced molecular weight of random combinations dictated by the reaction mechanism of enzymes involved, the substrate composition and molecular weight, and the often diffusion limited bioaggregate environment of the reaction. Based on a combination of empirical evidence, and model complexity reduction arguments, hydrolysis products are simplified to the respective mono- and oligomers of the polymers in a single reaction stoichiometry [49]. Along with disintegration, it is often the slowest and hence the rate-limiting step of the entire anaerobic digestion process [62,63]. The rate limitation is mechanistically linked to the diffusion-controlled physical contact between substrate particles and the hydrolytic enzymes. The majority of extracellular enzymes, but not all, are bound to, or retained by the bacteria, and therefore hydrolysis rates are directly proportional to the density of active bacteria. This has also been compared by direct observations of particulate cellulose-degrading consortia (Wang et al. (2011) [64]). For particulate substrates, this means that the rate-controlling factor is dictated by the contact area to the active bacteria, or by the adsorption kinetics of soluble enzymes to the same surface [65]. This resembles the general reaction kinetic model of surface catalyzed reactions, a process which for biocatalyzed reactions usually is described by Contois kinetics [66–69]. In contrast to the direct growth Contois model, hydrolysis in the activated sludge models and the ADM1 is a separate process leading to substrates for the bacterial growth process. While Contois kinetics is implemented in ASM, pseudo-first-order kinetics with respect to particulate substrates is used in ADM1 [65,69], which is a high biomass to substrate particle extreme. Specific rates can be determined using batch reactor tests: Biomethane potential (BMP) and hydrolysis rate constant (K_h) can be obtained by performing data fitting from batch reactor data, assuming hydrolysis to be the rate-limiting step. Batstone et al. [49] provide the simplest and most common hydrolysis rate expressions for biopolymers as follows:

$$\frac{dX}{dt} = K_h X, \tag{1a}$$

$$\frac{dX_{ch}}{dt} = K_{h,ch} X_{ch}, \tag{1b}$$

$$\frac{dX_{pr}}{dt} = K_{h,pr} X_{pr}, \tag{1c}$$

$$\frac{dX_{li}}{dt} = K_{h,li} X_{li}, \tag{1d}$$

where K_h is the pseudo-first-order hydrolysis rate constant in d^{-1}, X is particulate component in kg COD m^{-3}, and subscripts ch, pr, and li denote carbohydrate (polysaccharides), proteins, and lipids, respectively. This first-order kinetics is a special case of Vavilin's two-phase model (Vavilin's (1996) [65]) and the Contois model at high active biomass values, which, however, may not be the case during the initial batch test stage.

4.3. *Role of Microorganisms in the Hydrolysis of Particulates*

Anaerobic hydrolytic microorganisms carry out the process of breakdown of biopolymers into their respective monomers. There is a diverse group of hydrolytic microorganisms. Azman et al. [70] reported that the most abundant hydrolytic bacteria in biogas plants belong to the phylum Firmicutes and Bacteroidetes. Bacteria that belong to Phylum Fibrobacter, Spirochaetes, Thermotogae, and Chlorobi were also found, but less abundantly. Apart from some thermophilic hydrolytic bacteria such as Caldicellulosiruptor, most produce a multi-enzyme complex called Cellulosome. Cellulosome is an extracellular enzyme complex that is crucial in the adherence of bacterial cells onto surfaces, breakdown of macromolecules, and eventual absorption of soluble components into the cells. Lamed et al. [71] first described Cellulosome where they reported selective adherence of Clostridium thermocellum to cellulose particulates. All phyla containing anaerobic chemoheterotrophs may, however, be among hydrolytic bacteria to be found in anaerobic digesters. This can be investigated using a gene search for classical hydrolases to check whether these enzymes are widespread or not (not found in this survey) but in general (and for a diverse set of substrates) anything but a diverse hydrolytic bacteria community would be surprising. Microorganisms secrete a wide array of enzymes such as cellulase, protease, and lipase that facilitate hydrolysis. The mechanism of enzymatic action has been studied by several authors but gaps remain in the understanding of how enzyme-mediated hydrolysis occurs. According to Batstone et al. [49], the mechanism of release of enzymes can be carried out in three ways. The first is by directly releasing enzyme into the bulk liquid, the second way is bacteria attach to particles first and then release enzyme, and lastly, bacteria possess an enzyme that acts as a channel to the cell interior. Regardless of the mechanism of enzymatic release, transport, and diffusion of the enzyme to particles followed by reaction and enzyme deactivation occur. The description of hydrolysis in ADM1 is limited and a more general review on enzymes and enhancement of the biogas process is given by Parawira (2012) [72]. In addition to the description of cellulases for polysaccharides above, Ravndal and Kommedal (2017) [73] present a mechanistic description of starch degradation (from particulate to soluble polymers and final mineralization). Review by Jaeger et al. (1994) [74], Kanmani et al. (2015) [75], and Cammarota and Freire (2006) [76] for microbial lipases and esterases present further knowledge regarding mechanisms of enzymatic action. Temperature, pH, particulate size, and available surface area all influence hydrolysis. Various types of bacteria produce a wide range of enzymes each with its own optimal operational temperature; as a result, reports of optimum temperatures of hydrolytic microorganisms vary, but normally fall between 30 °C and 60 °C. Various studies have shown that the size of substrate particles and the rate of hydrolysis are inversely related [77–79]. It has also been observed that the amounts of particulates affect the microbial community [20]. Dai et al. [20] studied the effects of food waste TS on the microbial community composition at mesophilic conditions and observed changes in microbes involved in hydrolysis as well as methanogenesis.

4.4. Disintegration and Hydrolysis Models

Both disintegration and hydrolysis steps are extracellular processes. This is in contrast with the rest of anaerobic digestion processes of acidogenesis, acetogenesis and methanogenesis that are intracellular. Enzyme mediated breakdown of complex molecules into smaller ones occur during hydrolysis. Disintegration consists of mostly physical processes that result in the breakdown of solid composite particulates into smaller and easier-to-hydrolyze components. Understanding the kinetics of these processes is crucial in understanding the overall AD process. As rate-limiting steps, disintegration, and hydrolysis kinetics play a role in determining various AD parameters such as residence time and reactor size. Various attempts have been made to model disintegration and hydrolysis kinetics. A brief overview of some of the most applied models is provided below. First-order kinetics is the most common kinetics used to describe both disintegration and hydrolysis kinetics. Disintegration kinetics is provided in Equation (2) whereas Equations (1a)–(1d) show hydrolysis kinetics.

$$\frac{dX_C}{dt} = -K_{dis}X_c \tag{2}$$

where X_c is complex particulates in kgCOD m^{-3} and K_{dis} is disintegration rate constant in d^{-1}. First-order kinetics has been extensively used due to its simplicity but there are some drawbacks. First-order kinetics considers disintegration as a purely chemical process while it is partly a biological process that incorporates several other processes such as lysis and physical breakdown [80]. First-order kinetics does not account for the contribution of microorganisms in the disintegration process [80]. Therefore, models that use first-order kinetics such as ADM1 have limited precision when complex substrates such as manure are used. Both disintegration and hydrolysis are modeled by first-order kinetics in ADM1. Some modify the first-order kinetics by introducing a term to account for biological effects, such as Valentini et al. [81], who introduced the concentration of biomass into the hydrolysis kinetics equation as follows:

$$\frac{dX_C}{dt} = -K_hX_cX_{biom} \tag{3}$$

where K_h is hydrolysis rate constant. Varieties of this model, where the term X_{biom} in Equation (3) is replaced by X_{biom}^A('A-order biomass kinetics') by such as $X_{biom}^{1/2}$ has been proposed [82]. The value of A is between 0 and 1 and it depends on the particle's shape. Values of 0, 1/2 and 2/3 are proposed for flat, cylinder, and spherical shaped particles, respectively [78].

A two-phase model proposed the surface area of solid particulates covered by microorganisms to formulate kinetics of hydrolysis [51,65]. In the first phase, microbes colonize and cover all available surface of the particulates. In the second phase, the attached microbes release enzymes that progressively degrade the particulates at a constant depth per unit time. The two-phase model was developed based on the assumptions that:

- Particles are spherical;
- Hydrolysis rate is limited by the particle–bacteria contact area;
- Particle size > depth of bacterial layer;
- Number of particles per volume remains constant during hydrolysis;
- Size of particles decrease due to hydrolysis.

Based on these assumptions, Vavilin et al. [65] formulated a rate expression for particle degradation (Equation (4)) and an expression for a rate constant that is dependent on particle size and bacterial layer depth (Equation (5)).

$$X_h = K_h X_f^{1/3} X^{2/3} \tag{4}$$

$$K_h = 6K_{m,h} \frac{\rho_B}{\rho_X} \frac{\partial}{d_X} \tag{5}$$

where X_h is the rate of particle degradation in kgCOD m^{-3} d^{-1}, K_h is hydrolysis rate constant in d^{-1}, X_f is the concentration of influent biodegradable organic matter in kgCOD m^{-3}, X is the concentration of biodegradable suspended solids in kgCOD m^{-3}, $K_{m,h}$ is maximum specific hydrolysis rate in d^{-1}, ρ_B is the density of the microbial layer in kgCOD m^{-3}, ρ_X is the density of particulate in kgCOD m^{-3}, d_X is the current diameter of the hydrolyzed particle in m, and ∂ is the depth of the bacterial layer in m. The two-phase model shows a good fit for various types of substrates including swine manure [65]. A modified version of the Monod equation is sometimes used to model hydrolysis. It was first formulated for dissolved substrates. Because of that, there are critics who argue against using it for particle-rich substrates. However, Lin [83] showed, using anaerobic digestion of landfill leachate, that it can be applicable for substrates with suspended solids. The basic equation is given as follows:

$$\frac{dX_C}{dt} = -K_h \frac{X_c X_{biom}}{K_s + X_c} \tag{6}$$

where K_s is half-saturation concentration, X_C is complex particulates, K_h is hydrolysis rate constant, X_{biom} is active biomass.

Terashima and Lin [84] suggested a hydrolytic flux model based on the quantity of solid matter hydrolyzed per unit surface area per unit time. It is similar to the surface kinetic model suggested by Sanders et al. [85] (Equation (8)).

$$\frac{dX_C}{dt} = -K_h S_{surf} X_{biom} \rho_{solid} \tag{7}$$

$$\frac{dX_C}{dt} = -K_h S_{surf} \tag{8}$$

where S_{surf} is the surface area of solid particulates and ρ_{solid} is the density of solid particulates.

Various authors report that Contois kinetics provides a better fit for AD of complex substrates compared to first-order kinetics [86–88]. Contois kinetics (Equation (9)) takes effects of active biomass (X_{biom}) and its ratio to the slowly degradable substances (Xc) in the substrate into account.

$$\frac{dX_C}{dt} = -K_{m,dis} \frac{\frac{X_c}{X_{biom}}}{K_{s,dis} + \frac{X_c}{X_{biom}}} X_{biom} \tag{9}$$

where $K_{m,dis}$ is maximum disintegration rate in d^{-1}, $K_{s,dis}$ is a dimensionless half-saturation coefficient.

Dimock et al. [89] studied the influence of the size of protein particles on hydrolysis. They observed that hydrolysis results not only in the release of readily digested substrates but also in the break-up of large particles into smaller ones. The break-up increases the available surface area for hydrolysis. This shows that disintegration and hydrolysis cannot easily be distinguished (also justifying the way the two steps are presented together in this chapter). They suggested a surface-based particle break-up model (PBM) for disintegration and hydrolysis that takes into account the increase in the surface area due to the disintegration of large particles.

$$\frac{dX_C}{dt} = -K_h' f_{av} X_c = \rho_{PBM} \quad (10)$$

$$\frac{df_{av}}{dt} = c_{av}\rho_{PBM} \quad (11)$$

where K_h' is modified hydrolysis constant in m/d, f_{av} is surface to volume ratio variable in m^{-1}, c_{av} is a constant that correlates particle breakup and hydrolysis rates in m^2/kg. Studies on the influence of particle size of carbohydrates on the rate of hydrolysis and disintegration were also carried out by Kommedal et al. [90] who studied the effect of molecular weight of Dextrans (a form of polymeric carbohydrate) on microbial hydrolysis. They found that polymers of 6–500 kDa molecular weight range showed an inverse correlation to a half-order degradation rate expression (i.e., rate~M.Wt$^{-0.2}$).

$$\frac{dS_b}{dt} = -\frac{r_a A_f}{V} \quad (12)$$

$$r_A = K_{1/2,A}\sqrt{S_b - K_s \ln\left[\frac{K_s + S_b}{K_s}\right]} \quad (13)$$

where S_b is the polymer bulk-phase concentration in gTOC m^{-3} (TOC = total organic carbon), V is the bulk-phase reactor volume in m^3, r_A is areal removal rate in gTOC m^{-2} h^{-1}, $K_{1/2,\,A}$ is areal specific removal rate coefficient in $g^{1/2}$ $m^{-1/2}$ d^{-1}, K_s is Monod half-saturation coefficient in g/m^3, and A_f is the biofilm area in m^2. Particulate starch has also been used as a model substrate to investigate the degradation of suspended solids and colloids in aerobic granular sludge by de Kreuk et al. [91]. They studied the effect of particulate starch on granule morphology and overall conversion processes in aerobic granular sludge (sequencing batch reactor). They observed that the starch particles undergo fast adsorption onto granules followed by slow hydrolysis. Oxygen Uptake Rate (OUR) data indicated that particulate starch hydrolysis follows first-order kinetics (Equation (2)) as opposed to the zero-order kinetics observed when soluble starch was used. Soluble starch removal was similar both in aerobic and anaerobic conditions indicating that hydrolysis was independent of the presence of oxygen. Their results indicate that disintegration and not hydrolysis is the rate-limiting step in starch particle degradation. Presence of suspended solids in the influent also affected the morphology of aerobic granules, favoring the growth of filamentous structures on granule surfaces [92], suggesting active mechanisms to retain, and degrade feed particles in granular sludge processes (as also argued in Section 4.1). This mechanism of filamentous structures extending out of granules may also contribute to sludge loss, such as reported for slaughterhouse wastewater treatment [16], by a similar mechanism as sludge bulking in activated sludge processes [10].

5. Treatment Strategies for Particle-Rich Substrates

In earlier sections, we discussed problems associated with high-rate digestion of substrates with high suspended solids content. The main problem is usually the slow rate of particle degradation and as a result, excessive solids accumulation. This section reviews treatment strategies for particle-rich substrates, mainly focusing on enhancing the rate at which solid particulates are disintegrated and hydrolyzed. One of the most common strategies when dealing with particle-rich substrates is pretreatment. It has become an essential aspect of high-rate AD reactors with particle-rich substrates. Several methods of pretreatment discussed in earlier sections are based on reducing the size of the particulates in the substrate. Particle-rich substrates such as manure slurries produce a relatively high amount of biogas after pretreatment; however, economically sustainable pretreatment methods are limited. Many of the pretreatment methods assessed for the purpose of this article seem to add a significant amount to the overall cost (both capital and operational costs). Disintegration and hydrolysis within the AD reactors to limit cost are therefore the main topic here. Optimizing process parameters that affect disintegration and hydrolysis can have desired effects. Temperature, HRT, loading rate, and other process parameters have to be tuned but the main issue appears to be sludge retention. If the degradable feed particles can be retained much longer than the HRT, they can be degraded even if their degradation rates are low.

Several studies have shown promising results with regard to increased hydrolysis as well as increased biogas production due to the addition of enzymes [87]. Enhancing the degradation of lignocellulosic substances using enzymes that degrade lignin, cellulose, and other polysaccharides has been the focus of enzymatic treatment studies. Lignocellulosic materials constitute a significant percentage of solid particulates in manure slurries, food waste, and other substrates. Use of enzyme, combined with alkali, not only increases the yield but also the rate of production of biogas from such materials [88]. Microorganisms that are not usually associated with anaerobic digestion, such as fungi, may be used to break down lignocellulosic substances. Myint et al. [92] reported an increase in the hydrolysis of cattle manure using brown-rot fungi, which degrades cellulose and hemicellulosic substances. There are also reports of white-rot and soft-rot fungi being effective in degrading cellulose and lignin-based substrates [93]. There are indications that oxygen consuming facultative microorganisms can also be helpful in increasing hydrolysis through 'micro-aeration' or nitrate addition [54,61,94,95]. Research to enhance hydrolysis by a selection of microorganisms that can carry out fast and efficient hydrolysis is also carried out [96]. Anaerobic co-digestion of two or more complementary substrates is a strategy to alleviate problems associated with one substrate by adding another substrate that can improve the growth conditions for the entire AD microbial community. Co-digestion of particle-rich substrates such as manure slurries and industrial wastewater with low suspended solids content has been carried out extensively in the past few years, however, with moderate success [97]. Substrates with high solid content are associated with operational difficulties such as pumping problems like clogging, channeling, and mixing problems, indicating that operation and design of the reactor can affect not only hydrolysis but also the overall digestion process. Pumps used for high solid substrates must be able to operate under adverse conditions. Chopper pumps that are equipped with a cutting system could be a good fit for particle-rich substrates due to their contribution to particle size reduction and avoidance of clogging. The reactor design and configuration must be made in consideration with optimum solid hydrolysis.

Van Lier [98] stated that one way to treat suspended solids in high-rate reactors is to use separate reactor units such as clarifiers coupled to sludge digesters to enhance the digestion. In such an arrangement, the particle disintegration and hydrolysis steps are separately enhanced and hydrolyzed matter recycled to the main digester. An example of such a system is the UASB-digester system presented by Mahmoud et al. [99], where suspended solids are separated and transported to a separate digester with long retention time and high temperature for disintegration and hydrolysis before recycled to the main UASB reactor. Various other hybrid reactor systems have been proposed to enhance suspended solids removal such as up-flow anaerobic sludge bed fixed film (UASFF), hybrid anaerobic solid–liquid-UASB (HASL-UASB), and anaerobic filter-UASB [100], finding that such reactors are capable of treating substrates with a significant content of particulates and achieve high suspended solids removal. For example, Ahmad et al. [101] and Ohimain and Izah [102] show that UASB-type reactors could treat palm oil mill effluent with 50–60 g/L suspended solids content. Design features, such as reactors with recirculation that promote longer particle retention time, better contact, and solids removal should be considered when particle-rich substrates are used. Such strategies may, however, involve extra costs, while [11,23,25–27] show that conventional UASB-type reactors could treat high suspended solids content.

6. Concluding Remarks

This review paper assesses the state of high-rate digestion of particle-rich substrates. Successful high-rate AD of particle-rich substrates with TS content as high as 35% is possible as demonstrated by various authors. In conventional high-rate reactors such as UASB, the TS limit seemed to be lower with examples found at around 10% TS, above which mass transfer limitation becomes a problem. In many cases, some forms of reactor modification are applied and there may be HRT or OLR restrictions due to the high solid content of the substrates. We further conclude that:

- High-rate anaerobic digestion of particle-rich substrates has the potential to increase biogas production significantly due to the abundance of such substrates. However, economically sustainable methods of pretreatment are limited and several methods have been tried to improve the hydrolysis of solid particulates with varying degree of success (e.g., use of hydrolytic enzymes);
- Slow disintegration and hydrolysis of particulates is the main bottleneck in fully achieving the biogas potential of particle-rich substrates in high-rate sludge bed processes;
- Disintegration and hydrolysis of particulates within high-rate AD appear more promising;
- High-rate AD is traditionally assumed to only handle low particulate levels such as in industrial waste while newer studies show that high-rate reactors, especially hybrid types, may handle high levels of particulates;
- The degree of particle degradation within AD depends mainly on retention time so the challenge is to obtain long SRT in reactors with low HRT (SRT > HRT);
- Feed particles have typically much lower density than granular sludge and may therefore not be retained by the same reactor configurations as the granules. They may float when associated with biogas bubbles;
- Devices to retain floating sludge may, therefore, be required to obtain efficient disintegration and hydrolysis of particulates;
- There is evidence that the bacteria in the outer layer of granules can use extracellular polymeric structures to attach particles for the purpose of retaining and digesting feed particles;
- Disintegration and hydrolysis are treated as a single step in some models when they are both assumed to have first-order kinetics and this works well for non-complex substrates. Most particle-rich substrates are however quite complex and a wide range of models are proposed to handle such but more research is needed to find the best modeling approach. The relevance is emphasized by the fact that these are often the rate-limiting steps of the entire AD process on particle-rich substrates. However, modified first-order kinetics that classifies solid particles into fast and slow disintegrating fractions may be a good approach for particle-rich substrates since it retains the simplicity of first-order kinetics and improves on its accuracy.

Author Contributions: Conceptualization, All authors; methodology, F.A.T.; validation, F.A.T.; formal analysis, All authors; investigation, F.A.T.; resources, F.A.T.; data curation, F.A.T.; writing—original draft preparation, F.A.T.; writing—review and editing, All authors; visualization, F.A.T.; supervision, R.B. and W.H.B.; project administration, F.A.T. and R.B.; funding acquisition, R.B.

Funding: This research was part of a PhD project funded by Interreg BioGas2020 and partly funded by the Research Council of Norway ("Energyx project 269444 Bærekraftig biogass").

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bagi, Z.; Ács, N.; Böjti, T.; Kakuk, B.; Rákhely, G.; Strang, O.; Szuhaj, M.; Wirth, R.; Kovács, K.L. Biomethane: The Energy Storage, Platform Chemical and Greenhouse Gas Mitigation Target. *Anaerobe* **2017**, *46*, 13–22. [CrossRef] [PubMed]
2. Cantera, S.; Muñoz, R.; Lebrero, R.; López, J.C.; Rodríguez, Y.; García-Encina, P.A. Technologies for the Bioconversion of Methane into More Valuable Products. *Curr. Opin. Biotechnol.* **2018**, *50*, 128–135. [CrossRef] [PubMed]

3. Batstone, D.J.; Virdis, B. The Role of Anaerobic Digestion in the Emerging Energy Economy. *Curr. Opin. Biotechnol.* **2014**, *27*, 142–149. [CrossRef] [PubMed]
4. Boe, K.; Angelidaki, I. Serial CSTR Digester Configuration for Improving Biogas Production from Manure. *Water Res.* **2009**, *43*, 166–172. [CrossRef] [PubMed]
5. Berglann, H.; Krokann, K. *Biogassproduksjon På Basis Av Husdyrgjødsel—Rammebetingelser, Økonomi Og Virkemidler*; Norsk Institutt for Landbruksøkonomisk Forskning (NILF): Oslo, Norway, 2011.
6. Lettinga, G.; Hulshoff Pol, L.W. USAB-Process Design for Various Types of Wastewaters. *Water Sci. Technol.* **1991**, *24*, 87–107. [CrossRef]
7. Liu, Y.H.; He, Y.L.; Yang, S.C.; Li, Y.Z. The Settling Characteristics and Mean Settling Velocity of Granular Sludge in Upflow Anaerobic Sludge Blanket (UASB)-like Reactors. *Biotechnol. Lett.* **2006**, *28*, 1673–1678. [CrossRef] [PubMed]
8. Mahmoud, N.; Zeeman, G.; Gijzen, H.; Lettinga, G. Solids Removal in Upflow Anaerobic Reactors, a Review. *Bioresour. Technol.* **2003**, *90*, 1–9. [CrossRef]
9. Montoya AC, V.; Mazareli RD, S.; Da Silva, D.C.; De Oliveira, R.A.; Leite, V.D. Dairy Manure Wastewater in Serial UASB Reactors for Energy Recovery and Potential Effluent Reuse. *Braz. J. Chem. Eng.* **2017**, *34*, 971–983. [CrossRef]
10. Tchobanoglous, G.; Burton, F.L.; Stensel, H.D. *Wastewater Engineering Treatment and Reuse*; McGraw-Hill Higher Education: Boston, MA, USA, 2003.
11. Bergland, W.H.; Dinamarca, C.; Toradzadegan, M.; Nordgård, A.S.R.; Bakke, I.; Bakke, R. High Rate Manure Supernatant Digestion. *Water Res.* **2015**, *76*, 1–9. [CrossRef]
12. Lettinga, G. Sustainable Integrated Biological Wastewater Treatment. *Water Sci. Technol.* **1996**, *33*, 85–98. [CrossRef]
13. Lettinga GA, F.M.; Van Velsen AF, M.; Hobma, S.D.; De Zeeuw, W.; Klapwijk, A. Use of the Upflow Sludge Blanket (USB)Reactor Concept for Biological Wastewater Treatment, Especially for Anaerobic Treatment. *Biotechnol. Bioeng.* **1980**, *22*, 699–734. [CrossRef]
14. Rajeshwari, K.V.; Balakrishnan, M.; Kansal, A.; Lata, K.; Kishore, V.V.N. State-of-the-Art of Anaerobic Digestion Technology for Industrial Wastewater Treatment. *Renew. Sustain. Energy Rev.* **2000**, *4*, 135–156. [CrossRef]
15. Van Lier, J.B.; Van Der Zee, F.P.; Frijters, C.T.M.J.; Ersahin, M.E. Celebrating 40 Years Anaerobic Sludge Bed Reactors for Industrial Wastewater Treatment. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 681–702. [CrossRef]
16. Tilley, E.; Lüthi, C.; Morel, A.; Zurbrügg, C.; Schertenleib, R. *Compendium of Sanitation Systems and Technologies*, 2nd ed.; Swiss Federal Institute of Aquatic Science and Technology (Eawag): Dübendorf, Switzerland, 2014; pp. 121–122.
17. Zeeman, G.; Sanders, W.T.M.; Wang, K.Y.; Lettinga, G. Anaerobic Treatment of Complex Wastewater and Waste Activated Sludge–application of an Upflow Anaerobic Solid Removal (UASR) Reactor for the Removal and Pre-Hydrolysis of Suspended COD. *Water Sci. Technol.* **1997**, *35*, 121–128. [CrossRef]
18. Bergland, W.; Dinamarca, C.; Bakke, R. Efficient Biogas Production from the Liquid Fraction of Diary Manure. *Renew. Energy Power Qual. J.* **2014**, *12*. [CrossRef]
19. Bergland, W.; Dinamarca, C.; Bakke, R. Effects of Psychrophilic Storage on Manures as Substrate for Anaerobic Digestion. *BioMed Res. Int.* **2014**, *2014*, 712197. [CrossRef] [PubMed]
20. Yi, J.; Dong, B.; Jin, J.; Dai, X. Effect of Increasing Total Solids Contents on Anaerobic Digestion of Food Waste under Mesophilic Conditions: Performance and Microbial Characteristics Analysis. *PLoS ONE* **2014**, *9*, e102548. [CrossRef] [PubMed]
21. Fagbohungbe, M.O.; Dodd, I.C.; Herbert, B.M.J.; Li, H.; Ricketts, L.; Semple, K.T. High Solid Anaerobic Digestion: Operational Challenges and Possibilities. *Environ. Technol. Innov.* **2015**, *4*, 268–284. [CrossRef]
22. Abbassi-Guendouz, A.; Brockmann, D.; Trably, E.; Dumas, C.; Delgenès, J.-P.; Steyer, J.-P.; Escudié, R. Total Solids Content Drives High Solid Anaerobic Digestion via Mass Transfer Limitation. *Bioresour. Technol.* **2012**, *111*, 55–61. [CrossRef]
23. Massé, D.I.; Droste, R.L. Comprehensive Model of Anaerobic Digestion of Swine Manure Slurry in a Sequencing Batch Reactor. *Water Res.* **2000**, *34*, 3087–3106. [CrossRef]
24. Fujihira, T.; Seo, S.; Yamaguchi, T.; Hatamoto, M.; Tanikawa, D. High-Rate Anaerobic Treatment System for Solid/Lipid-Rich Wastewater Using Anaerobic Baffled Reactor with Scum Recovery. *Bioresour. Technol.* **2018**, *263*, 145–152. [CrossRef] [PubMed]

25. Andalib, M.; Hafez, H.; Elbeshbishy, E.; Nakhla, G.; Zhu, J. Treatment of Thin Stillage in a High-Rate Anaerobic Fluidized Bed Bioreactor (AFBR). *Bioresour. Technol.* **2012**, *121*, 411–418. [CrossRef] [PubMed]
26. Fang, C.; Sompong, O.; Boe, K.; Angelidaki, I. Comparison of UASB and EGSB Reactors Performance, for Treatment of Raw and Deoiled Palm Oil Mill Effluent (POME). *J. Hazard. Mater.* **2011**, *189*, 229–234. [CrossRef] [PubMed]
27. Borja, R.; Banks, C.J.; Sánchez, E. Anaerobic Treatment of Palm Oil Mill Effluent in a Two-Stage up-Flow Anaerobic Sludge Blanket (UASB) System. *J. Biotechnol.* **1996**, *45*, 125–135. [CrossRef]
28. Jensen, P.; Batstone, D. *Energy and Nutrient Analysis on Individual Waste Streams*; Meat and Livestock Australia Limited: North Sydney, Australia, 2012.
29. Lorimor, J.; Powers, W.; Sutton, A. *Manure Characteristics: Manure Management Systems Series: MWPS 18, Section 1*; MidWest Plan Service: Ames, IA, USA, 2004.
30. Wang, X.; Lu, X.; Li, F.; Yang, G. Effects of Temperature and Carbon-Nitrogen (C/N)Ratio on the Performance of Anaerobic Co-Digestion of Dairy Manure, Chicken Manure and Rice Straw: Focusing on Ammonia Inhibition. *PLoS ONE* **2014**, *9*, e97265. [CrossRef]
31. Hills, D.J. Effects of Carbon: Nitrogen Ratio on Anaerobic Digestion of Dairy Manure. *Agric. Wastes* **1979**, *1*, 267–278. [CrossRef]
32. Manitoba Agriculture. Food and Rural Development. Properties of Manure. Available online: https://www.gov.mb.ca/agriculture/environment/nutrient-management/pubs/properties-of-manure.pdf (accessed on 15 January 2019).
33. Hartmann, H.; Ahring, B.K. The Future of Biogas Production. In Proceedings of the Risø International Energy Conference, Risø, Denmark, 23 May 2005; pp. 23–25.
34. Moore, P.A.; Daniel, T.C.; Sharpley, A.N.; Wood, C.W. Poultry Manure Management: Environmentally Sound Options. *J. Soil Water Conserv.* **1995**, *50*, 321–327.
35. Hadin, Å.; Eriksson, O. Horse Manure as Feedstock for Anaerobic Digestion. *Waste Manag.* **2016**, *56*, 506–518. [CrossRef]
36. Kebreab, E.; Clark, K.; Wagner-Riddle, C.; France, J. Methane and Nitrous Oxide Emissions from Canadian Animal Agriculture: A Review. *Can. J. Anim. Sci.* **2006**, *86*, 135–157. [CrossRef]
37. Feng, L.; Ward, A.J.; Moset, V.; Møller, H.B. Methane Emission during On-Site Pre-Storage of Animal Manure Prior to Anaerobic Digestion at Biogas Plant: Effect of Storage Temperature and Addition of Food Waste. *J. Environ. Manag.* **2018**, *225*, 272–279. [CrossRef]
38. Hamilton, D.W.; Luce, W.G.; Heald, A.D. *Production and Characteristics of Swine Manure*; Oklahoma Cooperative Extension Service: Stillwater, MN, USA, 1997.
39. Jurado, E.; Antonopoulou, G.; Lyberatos, G.; Gavala, H.N.; Skiadas, I.V. Continuous Anaerobic Digestion of Swine Manure: ADM1-Based Modelling and Effect of Addition of Swine Manure Fibers Pretreated with Aqueous Ammonia Soaking. *Appl. Energy* **2016**, *172*, 190–198. [CrossRef]
40. Møller, H.B.; Sommer, S.G.; Ahring, B.K. Methane Productivity of Manure, Straw and Solid Fractions of Manure. *Biomass Bioenergy* **2004**, *26*, 485–495. [CrossRef]
41. Yenigün, O.; Demirel, B. Ammonia Inhibition in Anaerobic Digestion: A Review. *Process Biochem.* **2013**, *48*, 901–911. [CrossRef]
42. Kovács, E.; Wirth, R.; Maróti, G.; Bagi, Z.; Rákhely, G.; Kovács, K.L. Biogas Production from Protein-Rich Biomass: Fed-Batch Anaerobic Fermentation of Casein and of Pig Blood and Associated Changes in Microbial Community Composition. *PLoS ONE* **2013**, *8*, e77265. [CrossRef] [PubMed]
43. Molnar, L.; Bartha, I. High Solids Anaerobic Fermentation for Biogas and Compost Production. *Biomass* **1988**, *16*, 173–182. [CrossRef]
44. Kaparaju, P.; Rintala, J. Anaerobic Co-Digestion of Potato Tuber and Its Industrial by-Products with Pig Manure. *Resour. Conserv. Recycl.* **2005**, *43*, 175–188. [CrossRef]
45. Nuchdang, S.; Phalakornkule, C. Anaerobic Digestion of Glycerol and Co-Digestion of Glycerol and Pig Manure. *J. Environ. Manag.* **2012**, *101*, 164–172. [CrossRef]
46. Aangelidaki, I.; Ahrin, B.K.; Deng, H.; Schmidt, J.E. Anaerobic Digestion of Olive Oil Mill Effluents Together with Swine Manure in UASB Reactors. *Water Sci. Technol.* **2002**, *45*, 213–218. [CrossRef]
47. Lo, K.V.; Liao, P.H.; Gao, Y.C. Anaerobic Treatment of Swine Wastewater Using Hybrid UASB Reactors. *Bioresour. Technol.* **1994**, *47*, 153–157. [CrossRef]

48. Nordgård, A.S.R.; Bergland, W.H.; Vadstein, O.; Mironov, V.; Bakke, R.; Østgaard, K.; Bakke, I. Anaerobic Digestion of Pig Manure Supernatant at High Ammonia Concentrations Characterized by High Abundances of Methanosaeta and Non-Euryarchaeotal Archaea. *Sci. Rep.* **2017**, *7*, 15077. [CrossRef]
49. Batstone, D.J.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S.V.; Pavlostathis, S.G.; Rozzi, A.; Sanders, W.T.; Siegrist, H.; Vavilin, V.A. The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Sci. Technol.* **2002**, *45*, 65–73. [CrossRef] [PubMed]
50. Polizzi, C.; Alatriste-Mondragón, F.; Munz, G. Modeling the Disintegration Process in Anaerobic Digestion of Tannery Sludge and Fleshing. *Front. Environ. Sci.* **2017**, *5*, 37. [CrossRef]
51. Vavilin, V.A.; Fernandez, B.; Palatsi, J.; Flotats, X. Hydrolysis Kinetics in Anaerobic Degradation of Particulate Organic Material: An Overview. *Waste Manag.* **2008**, *28*, 939–951. [CrossRef] [PubMed]
52. Ariunbaatar, J.; Panico, A.; Esposito, G.; Pirozzi, F.; Lens, P.N.L. Pretreatment Methods to Enhance Anaerobic Digestion of Organic Solid Waste. *Appl. Energy* **2014**, *123*, 143–156. [CrossRef]
53. Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of Lignocellulosic Biomass for Enhanced Biogas Production. *Prog. Energy Combust. Sci.* **2014**, *42*, 35–53. [CrossRef]
54. Fletcher, M. Bacterial biofilms and biofouling. *Curr.Opin. Biotechnol.* **1994**, *5*, 302–306. [CrossRef]
55. Lew, B.; Belavski, M.; Admon, S.; Tarre, S.; Green, M. Temperature Effect on UASB Reactor Operation for Domestic Wastewater Treatment in Temperate Climate Regions. *Water Sci. Technol.* **2003**, *48*, 25–30. [CrossRef] [PubMed]
56. Uemura, S.; Harada, H. Treatment of Sewage by a UASB Reactor under Moderate to Low Temperature Conditions. *Bioresour. Technol.* **2000**, *72*, 275–282. [CrossRef]
57. Lew, B.; Tarre, S.; Belavski, M.; Green, M. UASB Reactor for Domestic Wastewater Treatment at Low Temperatures: A Comparison between a Classical UASB and Hybrid UASB-Filter Reactor. *Water Sci. Technol.* **2004**, *49*, 295–301. [CrossRef]
58. Ganidi, N.; Tyrrel, S.; Cartmell, E. Anaerobic Digestion Foaming Causes—A Review. *Bioresour. Technol.* **2009**, *100*, 5546–5554. [CrossRef]
59. Pagilla, K.R.; Craney, K.C.; Kido, W.H. Causes and Effects of Foaming in Anaerobic Sludge Digesters. *Water Sci. Technol.* **1997**, *36*, 463–470. [CrossRef]
60. Dictionary by Merriam-Webster: America's Most-Trusted Online Dictionary. Available online: https://www.merriam-webster.com/ (accessed on 15 February 2019).
61. Morgenroth, E.; Kommedal, R.; Harremoës, P. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment-a review. *Water Sci. Technol.* **2002**, *45*, 25–40. [CrossRef] [PubMed]
62. Noike, T.; Endo, G.; Chang, J.; Yaguchi, J.; Matsumoto, J. Characteristics of Carbohydrate Degradation and the Rate-limiting Step in Anaerobic Digestion. *Biotechnol. Bioeng.* **1985**, *27*, 1482–1489. [CrossRef] [PubMed]
63. Ma, J.; Frear, C.; Wang, Z.W.; Yu, L.; Zhao, Q.; Li, X.; Chen, S. A Simple Methodology for Rate-Limiting Step Determination for Anaerobic Digestion of Complex Substrates and Effect of Microbial Community Ratio. *Bioresour. Technol.* **2013**, *134*, 391–395. [CrossRef] [PubMed]
64. Wang, Z.W.; Lee, S.H.; Elkins, J.G.; Morrell-Falvey, J.L. Spatial and temporal dynamics of cellulose degradation and biofilm formation by Caldicellulosiruptor obsidiansis and Clostridium thermocellum. *AMB Express* **2011**, *1*, 1–10. [CrossRef]
65. Vavilin, V.A.; Rytov, S.V.; Lokshina, L.Y. A Description of Hydrolysis Kinetics in Anaerobic Degradation of Particulate Organic Matter. *Bioresour. Technol.* **1996**, *56*, 229–237. [CrossRef]
66. Contois, D.E. Kinetics of bacterial growth: Relationship between population density and specific growth rate of continuous cultures. *J. Gen. Microbiol.* **1959**, *21*, 40–50. [CrossRef]
67. Henze, M.; Gujer, W.; Mino, T.; Van Loosdrecht, M. *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*; IWA Publishing: London, UK, 2000.
68. Wang, Z.W.; Li, Y. A theoretical derivation of the Contois equation for kinetic modeling of the microbial degradation of insoluble substrates. *Biochem. Eng. J.* **2014**, *82*, 134–138. [CrossRef]
69. Koch, K.; Drewes, J.E. Alternative Approach to Estimate the Hydrolysis Rate Constant of Particulate Material from Batch Data. *Appl. Energy* **2014**, *120*, 11–15. [CrossRef]
70. Azman, S.; Khadem, A.F.; Van Lier, J.B.; Zeeman, G.; Plugge, C.M. Presence and Role of Anaerobic Hydrolytic Microbes in Conversion of Lignocellulosic Biomass for Biogas Production. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 2523–2564. [CrossRef]

71. Bayer, E.A.; Kenig, R.; Lamed, R. Adherence of Clostridium Thermocellum to Cellulose. *J. Bacteriol.* **1983**, *156*, 818–827. [CrossRef] [PubMed]
72. Parawira, W. Enzyme research and application in biotechnological intensification of biogas production. *Crit. Rev. Biotechnol.* **2012**, *32*, 172–186. [CrossRef] [PubMed]
73. Ravndal, K.T.; Kommedal, R. Starch degradation and intermediate dynamics in flocculated and dispersed microcosms. *Water Sci. Technol.* **2017**, *76*, 2928–2940. [CrossRef] [PubMed]
74. Jaeger, K.E.; Ransac, S.; Dijkstra, B.W.; Colson, C.; Van Heuvel, M.; Misset, O. Bacterial lipases. *FEMS Microbial. Rev.* **1994**, *15*, 29–63. [CrossRef] [PubMed]
75. Kanmani, P.; Aravind, J.; Kumaresan, K. An insight into microbial lipases and their environmental facet. *Int. J. Environ. Sci. Technol.* **2015**, *12*, 1147–1162. [CrossRef]
76. Cammarota, M.C.; Freire, D.M.G. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresour. Technol.* **2006**, *97*, 2195–2210. [CrossRef] [PubMed]
77. Dasari, R.K.; Berson, R.E. The Effect of Particle Size on Hydrolysis Reaction Rates and Rheological Properties in Cellulosic Slurries. In *Applied Biochemistry and Biotecnology*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 289–299.
78. Yeh, A.-I.; Huang, Y.-C.; Chen, S.H. Effect of Particle Size on the Rate of Enzymatic Hydrolysis of Cellulose. *Carbohydr. Polym.* **2010**, *79*, 192–199. [CrossRef]
79. Ramirez, I.; Mottet, A.; Carrère, H.; Déléris, S.; Vedrenne, F.; Steyer, J.-P. Modified ADM1 Disintegration/Hydrolysis Structures for Modeling Batch Thermophilic Anaerobic Digestion of Thermally Pretreated Waste Activated Sludge. *Water Res.* **2009**, *43*, 3479–3492. [CrossRef]
80. Rozzi, A.; Merlini, S.; Passino, R. Development of a Four Population Model of the Anaerobic Degradation of Carbohydrates. *Environ. Technol.* **1985**, *6*, 610–619. [CrossRef]
81. Valentini, A.; Garuti, G.; Rozzi, A.; Tilche, A. Anaerobic Degradation Kinetics of Particulate Organic Matter: A New Approach. *Water Sci. Technol.* **1997**, *36*, 239–246. [CrossRef]
82. Aldin, S. *The Effect of Particle Size on Hydrolysis and Modeling of Anaerobic Digestion*; Electronic Thesis and Dissertation Repository: Western Ontario, ON, Canada, 2010; p. 60.
83. Lin, C.Y. Anaerobic Digestion of Landfill Leachate. *Water SA* **1991**, *17*, 301–306.
84. Terashima, Y.; Lin, S. On the Modeling of Microbiological Hydrolysis of Organic Solids. *Water Sci. Technol.* **2000**, *42*, 11–19. [CrossRef]
85. Sanders, W.T.M.; Geerink, M.; Zeeman, G.; Lettinga, G. Anaerobic hydrolysis kinetics of particulate substrates. *Water Sci. Technol.* **2000**, *41*, 17–24. [CrossRef] [PubMed]
86. Hashimoto, A.G.; Chen, Y.R.; Varel, V.H. Theoretical Aspects of Methane Production: State-of-the-Art. In *Livestock Waste, a Renewable Resource*; American Society of Agricultural Engineers: St. Joseph, Michigan, USA, 1981; pp. 86–91.
87. Speda, J.; Johansson, M.A.; Odnell, A.; Karlsson, M. Enhanced Biomethane Production Rate and Yield from Lignocellulosic Ensiled Forage Ley by in Situ Anaerobic Digestion Treatment with Endogenous Cellulolytic Enzymes. *Biotechnol. Biofuels* **2017**, *10*, 129. [CrossRef] [PubMed]
88. Liu, X.; Zicari, S.M.; Liu, G.; Li, Y.; Zhang, R. Pretreatment of Wheat Straw with Potassium Hydroxide for Increasing Enzymatic and Microbial Degradability. *Bioresour. Technol.* **2015**, *185*, 150–157. [CrossRef] [PubMed]
89. Dimock, R.; Morgenroth, E. The Influence of Particle Size on Microbial Hydrolysis of Protein Particles in Activated Sludge. *Water Res.* **2006**, *40*, 2064–2074. [CrossRef] [PubMed]
90. Kommedal, R.; Milferstedt, K.; Bakke, R.; Morgenroth, E. Effects of Initial Molecular Weight on Removal Rate of Dextran in Biofilms. *Water Res.* **2006**, *40*, 1795–1804. [CrossRef]
91. De Kreuk, M.K.; Kishida, N.; Tsuneda, S.; Van Loosdrecht, M.C.M. Behavior of Polymeric Substrates in an Aerobic Granular Sludge System. *Water Res.* **2010**, *44*, 5929–5938. [CrossRef]
92. Myint, M.T.; Nirmalakhandan, N. Enhancing Anaerobic Hydrolysis of Cattle Manure in Leachbed Reactors. *Bioresour. Technol.* **2009**, *100*, 1695–1699. [CrossRef]
93. Hatakka, A.I. Pretreatment of Wheat Straw by White-Rot Fungi for Enzymic Saccharification of Cellulose. *Eur. J. Appl. Microbiol. Biotechnol.* **1983**, *18*, 350–357. [CrossRef]
94. Montalvo, S.; Vielma, S.; Borja, R.; Huiliñir, C.; Guerrero, L. Increase in Biogas Production in Anaerobic Sludge Digestion by Combining Aerobic Hydrolysis and Addition of Metallic Wastes. *Renew. Energy* **2018**, *123*, 541–548. [CrossRef]

95. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, DC, USA, 1999; p. 1325, ISBN 978-08-7553-235-6.
96. de Lourdes Moreno, M.; Pérez, D.; García, M.T.; Mellado, E. Halophilic Bacteria as a Source of Novel Hydrolytic Enzymes. *Life* **2013**, *3*, 38–51. [CrossRef] [PubMed]
97. Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M.S.; Fonoll, X.; Peces, M.; Astals, S. A Critical Review on Anaerobic Co-Digestion Achievements between 2010 and 2013. *Renew. Sustain. Energy Rev.* **2014**, *36*, 412–427. [CrossRef]
98. Van Lier, J.B. High-Rate Anaerobic Wastewater Treatment: Diversifying from End-of-the-Pipe Treatment to Resource-Oriented Conversion Techniques. *Water Sci. Technol.* **2008**, *57*, 1137–1148. [CrossRef] [PubMed]
99. Mahmoud, N.; Zeeman, G.; Gijzen, H.; Lettinga, G. Anaerobic Sewage Treatment in a One-Stage UASB Reactor and a Combined UASB-Digester System. *Water Res.* **2004**, *38*, 2348–2358. [CrossRef] [PubMed]
100. Tauseef, S.M.; Abbasi, T.; Abbasi, S.A. Energy Recovery from Wastewaters with High-Rate Anaerobic Digesters. *Renew. Sustain. Energy Rev.* **2013**, *19*, 704–741. [CrossRef]
101. Ahmad, A.; Ghufran, R.; Abd Wahid, Z. Effect of COD Loading Rate on an Upflow Anaerobic Sludge Blanket Reactor during Anaerobic Digestion of Palm Oil Mill Effluent with Butyrate. *J. Environ. Eng. Landsc. Manag.* **2012**, *20*, 256–264. [CrossRef]
102. Ohimain, E.I.; Izah, S.C. A Review of Biogas Production from Palm Oil Mill Effluents Using Different Configurations of Bioreactors. *Renew. Sustain. Energy Rev.* **2017**, *70*, 242–253. [CrossRef]