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Review

Influence of Pre-Hydrolysis on Sewage Treatment in an Up-Flow Anaerobic Sludge BLANKET (UASB) Reactor: A Review

Rajinikanth Rajagopal [1,*], Mahbuboor Rahman Choudhury [2], Nawrin Anwar [2], Bernard Goyette [1] and Md. Saifur Rahaman [2]

[1] Sherbrooke Research and Development Center, Agriculture and Agri-Food Canada, 2000 College Street, Sherbrooke, QC J1M 0C8, Canada; Bernard.goyette@canada.ca

[2] Department of Building, Civil, and Environmental Engineering, Concordia University, 1515 Saint-Catherine St W, Montreal, QC H3G 2W1, Canada; mahbuboor@gmail.com (M.R.C.); n_anwar@encs.concordia.ca (N.A.); saifur.rahaman@concordia.ca (M.S.R.)

* Correspondence: rajinikanth.rajagopal@canada.ca; Tel.: +1-819-780-7132

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Abstract: The up-flow anaerobic sludge blanket (UASB) process has emerged as a promising high-rate anaerobic digestion technology for the treatment of low- to high-strength soluble and complex wastewaters. Sewage, a complex wastewater, contains 30–70% particulate chemical oxygen demand (COD_P). These particulate organics degrade at a slower rate than the soluble organics found in sewage. Accumulation of non-degraded suspended solids can lead to a reduction of active biomass in the reactor and hence a deterioration in its performance in terms of acid accumulation and poor biogas production. Hydrolysis of the COD_P in sewage prior to UASB reactor will ensure an increased organic loading rate and better UASB performance. While single-stage UASB reactors have been studied extensively, the two-phase full-scale treatment approach (i.e., a hydrolysis unit followed by an UASB reactor) has still not yet been commercialized worldwide. The concept of treating sewage containing particulate organics via a two-phase approach involves first hydrolyzing and acidifying the volatile suspended solids without losing carbon (as methane) in the first reactor and then treating the soluble sewage in the UASB reactor. This work reviews the available literature to outline critical findings related to the treatment of sewage with and without hydrolysis before the UASB reactor.

Keywords: anaerobic digestion; hydrolysis; methanogens; sewage; up-flow anaerobic sludge blanket reactor; volatile fatty acids

1. Introduction

Domestic sewage is considered a complex wastewater, as it contains both particulate and dissolved organics. About 30–70% of the particulate chemical oxygen demand (COD_P) of domestic sewage is found in the form of organic polymers such as carbohydrates, lipids, and proteins. These particulate organics, which have slow degradation kinetics, can diminish the performance of treatment processes. Treatment of sewage by conventional approaches, including primary sedimentation and secondary aerobic biological treatment, is very effective. However, this efficiency comes at high capital and operational costs, as well as advanced technology requirements [1,2]. High-rate anaerobic digestion biotechnology has proven to be an excellent process and is considered by many authors to be the core of sustainable waste management techniques [1,3–7].

The up-flow anaerobic sludge blanket (UASB) digester is one such high-rate anaerobic system that has been extensively studied and adopted all over the world in laboratory, pilot-scale, and full-scale implementations [8]. Compared to aerobic processes, high-rate anaerobic sewage treatment

processes offer: (a) high removal efficiency in the system, even at high organic loading rates (OLR) and short hydraulic retention times (HRT); (b) simpler reactor construction and operation; (c) flexibility in terms of design scale; (d) a net-positive energy producing process through the production of high quality renewable fuel in the form of biogas; (e) lower sludge production rates with well stabilized sludge production for final disposal with good dewatering characteristics (due to the slow growth rate of anaerobic microorganisms); and (f) low nutrient and chemical requirements [3,5,6,9,10]. While these comparisons show the positive aspects of anaerobic process, it should be kept in mind that comparison of aerobic and anaerobic processes should be based on the type of wastewater. Anaerobic treatment processes have been found to be advantageous for very high strength sewage treatment. Despite all these advantages, there exist some drawbacks to the application of high-rate anaerobic treatment processes. These limitations include: long solids retention time in the reactors, long start up time requirement, impure biogas generation possibly leading to bad odors, incomplete or insufficient removal of organic matter, pathogens and nutrients in the final effluent, and necessity of further post-treatment to meet discharge or reuse standards [3,5,6,9,10].

Anaerobic digestion of complex wastewater is a multistep process involving microorganisms and occurring in the absence of oxygen. The interactions between the microbial community takes place in a series and parallel reactions that degrade complex polymers like carbohydrates, proteins, nucleic acids, and lipids, into methane (CH_4) and carbon dioxide (CO_2) [11]. At a molecular level, these steps occur in sequence; however, in a reactor they appear to progress simultaneously. Several groups of microorganisms present in reactors catalyze reactions occurring in the anaerobic digestion process. These are fermentative bacteria, hydrogen producing acetogenic microorganisms, hydrogen-consuming acetogenic microorganisms, CO_2 reducing methanogens, and aceticlastic methanogens. A schematic of the processes of anaerobic digestion is presented in Figure 1, which shows the reaction pathways in anaerobic digestion along with the catalytic microorganisms.

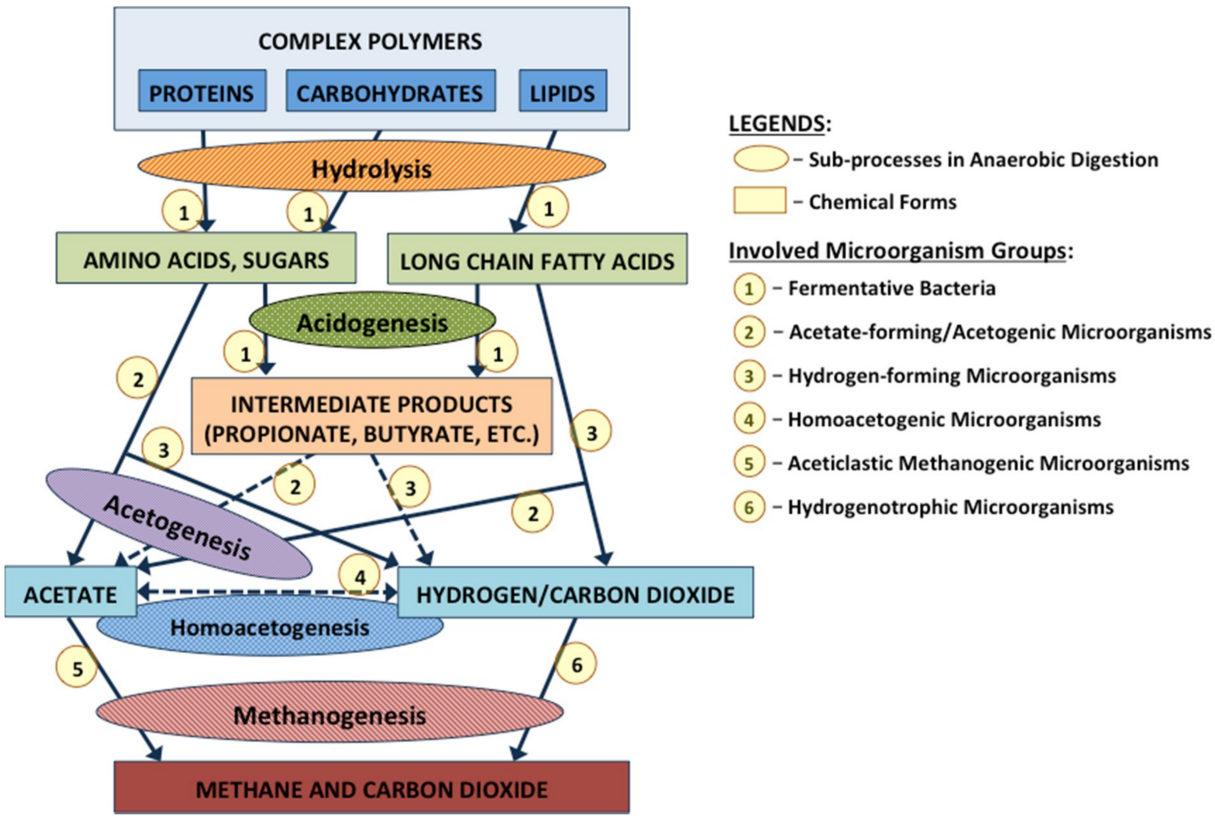

Figure 1. Anaerobic digestion of organic polymeric materials indicating sub-processes and involved bacterial groups (Figure adapted from Seghezzo, 2004 [11]).

Due to the limitations of anaerobic treatment and UASB technology in the treatment of sewage, researchers are continuously working to enhance the performance of anaerobic digesters by changing reactor configurations [1,6,12]; enhancing start-up and granulation processes in the reactors by using multivalent cations [13–16], natural polymers [17,18], or synthetic polymers [19,20]; and incorporating complementary post-treatment schemes such as activated sludge [21,22] or sequencing batch reactors [23,24] to treat the effluent produced by UASB. Various studies have shown that accumulation of suspended solids in sewage adversely affects the anaerobic digestion process [3,11,25–27]. These solids decrease sludge activity due to adsorption and entrapment, limit substrate transfer, lead to the formation of a "scum" layer, inhibit granulation, and increase sludge volume, which in turn requires frequent emptying of the reactor [1]. The relatively slow digestion of organic solids results in high accumulation in the reactors, especially at lower temperatures (<20 °C, i.e., psychrophilic range). As a result, the solid retention time is decreased and the performance of UASB reactors is also reduced [4,27–30]. Both gas production rate and chemical oxygen demand (COD) removal drop significantly when the temperature drops to the psychrophilic range [31]. Several reactor modification strategies have been adopted to incorporate pre-hydrolysis of wastewater prior to UASB, which should increase the digester OLR and improve the performance of UASB. Such reactor modifications involve applying a two-stage anaerobic process, which results in the entrapment of particulate organic matter and its partial hydrolysis into soluble compounds in the first stage and these pre-hydrolyzed organics are then digested in the second stage. The removal efficiency of suspended solids in the first reactor will be higher than that of organic matter and excess sludge needs to be discharged regularly. As a result, of that, the sludge age remains relatively low in this reactor, hindering the development of the slow-growing methanogens and reducing methanogenesis to a minimum. Two examples of such first-stage reactors include Hydrolysis Up-flow Sludge Blanket (HUSB) reactors and Up-flow Anaerobic Solids Removal (UASR) reactors. Incorporation of HUSB reactor achieved over 50% hydrolysis of the removed suspended solids at higher ambient temperatures (exceeding 19 °C) [32]. The HUSB reactor can be considered as a relatively highly loaded UASB system for the removal and hydrolysis of suspended COD. The hydraulic retention time in the HUSB reactor is very similar to that applied in primary sedimentation tanks, but the removal efficiencies of COD, BOD, and suspended solids are considerably higher [32]. In UASR, only suspended solids removal is obtained, as in normal settling tanks [33], while in HUSB reactors, hydrolysis also takes place. Therefore, more sludge has to be discharged from UASR than from HUSB reactors [5].

The objective of this study was to review the effect of pre-hydrolysis on the treatment of complex sewage in an UASB reactor. The review reports in the performance of UASB reactors (with and without pre-hydrolysis) from the literature and makes a comparison between the treatment efficiencies of the anaerobic digestion systems in terms of solids reduction, removal of various COD fractions, and sludge production. Finally, the review provides some perspectives for future research requirements regarding complex wastewater treatment in a two-phase hydrolysis-UASB approach.

2. Sewage Treatment in High-Rate Anaerobic Systems

High-rate anaerobic processes for treatment of complex wastewater affects sludge retention time (SRT) and hydraulic retention time (HRT) in the system [34]. As more biomass concentration accumulates in the system, the wastewater treatment process allows for relatively low hydraulic retention times. The bulk liquid phase invariably flows through the system with little impedance. In this case, the SRT/HRT ratio is greater than that of conventional anaerobic treatment technologies. The value of SRT/HRT depends on how well the system is able to retain biomass. The success of the high-rate anaerobic treatment system is, in part, due to the application of a relatively high loading rate, maintaining long SRTs at relatively short HRTs due to sludge immobilization [35,36].

As mentioned earlier, anaerobic digestion of complex wastewater involves a series of reactions catalyzed by several groups of microorganisms in the absence of oxygen (Figure 1). The principal reaction sequences have been classified into the following groups: (a) Hydrolysis,

(b) Fermentation, (c) Acidogenesis, (d) Acetogenesis/Dehydrogenation, and (e) Methanogenesis [37–40]. In an initial exoenzyme-catalyzed reaction, composite biopolymers are hydrolyzed to soluble mono, di, or oligomers [41]. The complex particulate materials are converted into carbohydrates, proteins, and lipids, and later these complex organic compounds are transformed into corresponding monomers (like amino acids, sugars, and long chain fatty acids, etc.). This step, commonly referred to as 'hydrolysis', is the rate-limiting step in the overall anaerobic treatment processes for wastes containing lipids and/or substantial amount of particulate matter [42,43]. Intracellular enzymes in acidogenic microorganisms induce fermentation of these soluble fractions to reduced organic compounds like short chain fatty acids, alcohols, and lactate in a process known as acidogenesis [38]. The hydrolyzed amino acids, sugars, and long chain fatty acids are taken up by the acidogenic microorganisms and fermented, resulting in the production of formate, propionate, butyrate, lactate, etc. If fatty acid isomers are produced, they are mainly derived as a result of hydrolysis of lipids and amino acids, which are produced as a result of protein hydrolysis. Acetogenic microorganisms further oxidize fatty acids and the resulting cleavage products (CO_2/H_2 formed by hydrogen-forming microorganisms or acetate formed by acetate-forming microorganisms) can be taken up by methanogens and be converted to CH_4 and CO_2. Lactate is oxidized to pyruvate, which is decarboxylated to yield acetate, CO_2, and H_2. If ethanol is present, it is oxidized to acetate and hydrogen, and the hydrogen is used for CO_2 reduction [41]. Acetate can also be formed via the CO_2/H_2 pathway in a step called homoacetogenesis. The methanogens are able to directly use substrates like H_2, acetate, formate and methanol to produce CH_4 [38]. To optimize the overall anaerobic digestion process, the rate-limiting hydrolysis process must be improved. To accommodate the rate-limiting hydrolysis of particulates in complex wastewater, a longer SRT is required depending on the applied process temperature [34]. The increased SRT ensures retention of slowly growing organisms even at relatively shorter HRTs, which ensures high OLR [1]. Different high-rate anaerobic systems have been developed, including the anaerobic filter [44], the up-flow anaerobic sludge blanket [45], the fluidized and expanded bed reactors [46], the down flow stationary fixed film reactor [47], and the anaerobic baffled reactors [48].

Several researchers have introduced and investigated optimized versions of the UASB system. These modified systems include the expanded granular sludge bed (EGSB) reactor [49], the UASB-septic tank reactor [50], the hydrolysis up-flow sludge bed (HUSB) reactor [32], the thermophilic up-flow staged sludge bed (USSB) reactor [51], the up-flow anaerobic solids removal (UASR) reactor [33], the hybrid EGSB-fixed bed reactor [52], the anaerobic bioreactor with a fixed-structure bed (ABFSB) [53], and the two-stage anaerobic filter/anaerobic hybrid (AF/AH) system [54]. While UASB and EGSB reactors have been identified as the most effective anaerobic treatment system for low strength wastewater [5], the modified anaerobic baffled reactor has also shown improved treatment performance [55].

3. Sewage Treatment in a UASB Reactor

The UASB process has been successfully implemented as a high-rate anaerobic technology for the treatment of low to high strength soluble wastewaters as well as complex wastewaters [56,57]. The up-flow anaerobic sludge blanket reactor (UASBR) schematic diagram, shown in Figure 2, indicates two parts in the reactor: (a) a vertical column, and (b) a gas-liquid-solid phase separator, which is placed in the upper section and divides the reactor into a lower (digestion zone) and an upper section (the settling zone) [58,59]. The sewage, introduced uniformly from the bottom of the reactor, passes through the sludge bed and enters into the settling zone via openings between the phase separator elements. One of the characteristic features of UASB is sludge granulation. During the process, anaerobic microorganisms agglomerate to form biogranules by the process of impulsive aggregation and form dense, compact granules with good settling characteristics [1,60]. The sludge granules form after a certain period (usually 2–8 months), depending on the operating conditions, wastewater characteristics, and seed sludge. Elmitwalli (2000) reported this long startup period as one of the main drawbacks of anaerobic treatment of domestic sewage in high-rate systems

when seed sludge is not available due to low growth rate of methanogenic microorganisms [61]. Generally, UASBRs are inoculated with a suitable seed source to shorten the startup time [62].

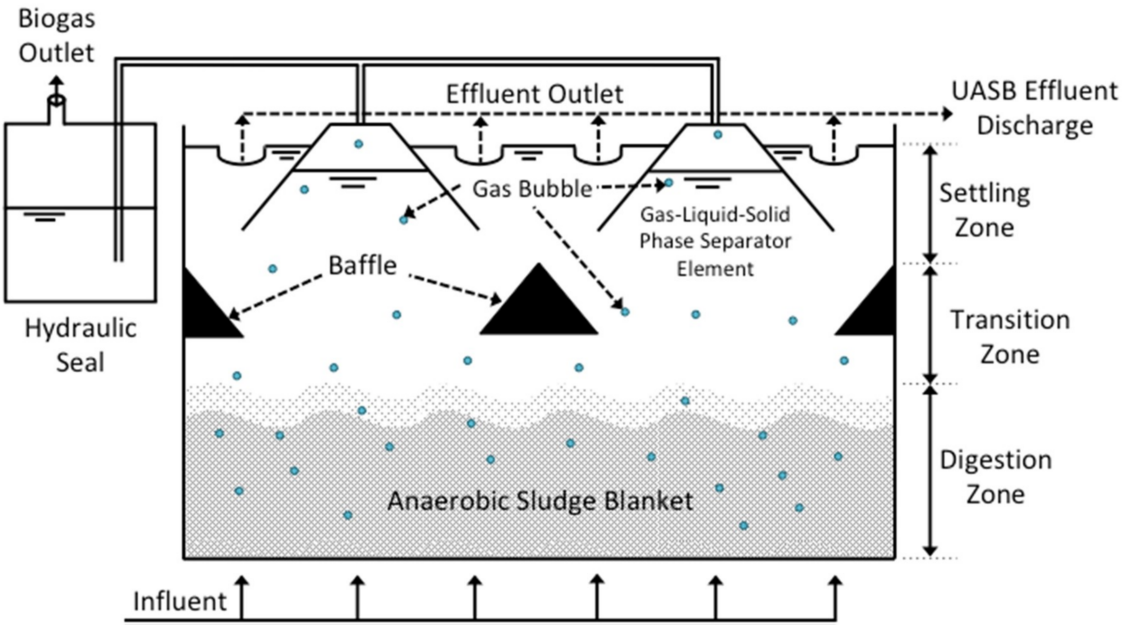

Figure 2. Schematic showing different components and zones of a UASBR system. (Figure reproduced following van Haandel and Lettinga 1994, [63]).

A dense sludge bed, having high settling properties, develops either with granular or flocculent features. In the case of sewage, which is a low- to medium-strength wastewater, flocculent sludge forms in the reactor. Above the dense sludge bed, a sludge blanket zone develops with a diffuse growth pattern and lower particle settling velocities [1]. Biological reactions take place in the sludge bed and sludge blanket region. As the wastewater passes through the biomass in the dense sludge bed and the sludge blanket region, the dissolved substrate is digested and the particulate organic matter is retained and digested to form biogas [61,64]. The produced biogas (which consists mostly CH_4 and CO_2) in the digestion section is captured by the phase separator setup so the gas bubbles cannot interfere with the solid settling. An air/gas pocket is located under the phase separator element to prevent solids from escaping through the gas outlet. As a result, sludge particles on the phase separator fall back into the digestion zone, and a large sludge mass is retained in the reactor [56,58]. Finally, an effluent with less suspended solids is discharged from the settling zone.

In comparison with other high-rate anaerobic digesters, UASB processes have the advantage of not requiring any support media for biomass attached growth or immobilization yet achieve high removal of COD through utilization of available granular or flocculent sludge [6,65]. The biomass of good settling properties is not prone to washout from the system under practical reactor conditions. The natural turbulence caused by influent up-flow and gas production helps to maintain adequate contact between biomass and wastewater. Like all other modern high-rate reactors, the UASBR is able to separate SRT/HRT through the use of the sludge blanket that develops as a result of granulation [66].

3.1. Sewage Treatment in a Single-Stage UASBR without Hydrolysis

3.1.1. Laboratory and Pilot-Scale Treatment

Application of basic UASB reactors for sewage treatment at low operational temperatures has been studied in the Netherlands since 1976 [67]. Since the early 1980s, considerable research and development has been carried out with respect to anaerobic municipal wastewater treatment systems and, specifically, UASB reactors [68]. Several studies have observed the application of

single-stage UASB process for sewage treatment in different laboratory- and pilot-scale settings. The pilot-scale UASB plant built in Cali, Columbia in 1982–1983 with support from the Netherlands was claimed to be the first of its kind in the world [69]. A 64-m^3 reactor was operated at an average sewage temperature of 25 °C. Diluted digested cow manure was used as inoculum, and the plant was fully operational after 6 months at an HRT of 8 h. COD and BOD removal efficiencies higher than 75% were observed while SS removal was about 70%. Tables 1 and 2 outline selected key operating parameters and performance of laboratory-scale (reactor size range 2–35 liters) and pilot-scale (reactor size range 55–2000 liters) UASB systems as studied by different researchers.

3.1.2. Full-Scale Treatment

Following the successful installation and operation of the first 64 m^3 pilot-scale municipal UASB in Cali, Columbia [10,70,71], there has been a rapid rise in the application of full-scale UASB plants for municipal sewage treatment, especially in tropical countries like Brazil, Mexico, and India. The results of the 64m^3 demonstration scale UASBR were so promising that the Ganga Project Directorate requested the demonstration of the UASB technology under Indian conditions in Kanpur. Based upon the results of this demonstration plant, full-scale plants would be constructed in Kanpur and Mirzapur [72]. This demonstration plant has been in operation since April 1989 in Kanpur. The UASBR has a volume of 1200 m^3 and the plant has a design capacity to treat 5000 m^3 of raw sewage per day. The startup period was about 10 weeks. COD, BOD and TSS removals of 74%, 75%, and 75%, were achieved, respectively, at a nominal HRT of 6 h.

Due to the Kanpur results, a full-scale UASB plant, followed by pond treatment, was implemented in Mirzapur, India, constructed as part of the Indo-Dutch Environmental and Sanitary Engineering Project under the Ganga Action Plan. This plant has been in full operation since April 1994 [72]. The overall removal efficiency of the Mirzapur wastewater treatment plant for COD, BOD and TSS was about 81, 86 and 89%, respectively [73,74].

Recently, a large number of full-scale UASBRs have entered operation in Europe, US and Japan, with more than 100 recently constructed plants found in in Japan, Brazil and India. Heffernan et al. (2011) identified over 45 municipal UASB plants in India designed for an average daily flow of 10,000 m^3 or more, and 15 such plants were identified in Brazil [75]. About 10 UASB-based sewage treatment plants have been commissioned within a distance of 50–300 km from Roorkee, India, with all the plants having the same sequence (i.e., screening, grit removal, UASB and post treatment by polishing ponds). HRT for each UASB reactors varies from 8.0–9.4 h. Average BOD and TSS removal has been found to vary from 78–89% and 78–93%, respectively [76]. Results of municipal sewage treatment in full-scale UASB reactors under different working conditions are presented in Table 3.

The various processes involved in the anaerobic degradation of sewage in a single-stage UASBR include: (a) Hydrolysis, (b) Fermentation, (c) Acidogenesis, (d) Acetogenesis/ dehydrogenation, and (e) Methanogenesis (as described previously). For sewage containing high amounts of particulate matter or lipids, the hydrolysis step is the rate-limiting step in the overall anaerobic treatment processes [42,43]. Some researchers have reported the methanogenesis step to be the rate-limiting step in the overall conversion of organic matter to CH_4 when treating domestic sewage in a one-stage UASBR [4]. Mahmoud et al. [4] reported that better methanogenic conditions in the reactor enhances the hydrolysis step by improving contact between the substrate and the hydrolytic enzymes due to biogas production and therefore the methanogenesis step becomes the rate-limiting step. The methanogenesis step is observed to be rate-limiting in cases where the digester influent is soluble in nature.

The performance of the UASBR depends on several factors such as: (a) characteristics of suspended solids, (b) reactor temperature, (c) organic loading rates, (d) hydraulic and solids retention times adopted in the reactor, and (e) feeding rate or up-flow velocity [5,77,78]. High suspended solids in the influent can lead to problems like frequent required desludging of the reactor, reduction in

viable sludge in the reactor due to reduced SRT, reduction in sludge activity due to accumulation of non-viable solids, sludge lifting and sludge washout, and required disposal of non-viable sludge from filter beds. There exist few, if any, differences between flocculent and granular sludge bed UASB reactors with respect to the applicable OLRs, when a high total suspended solids (TSS) removal efficiency should be accomplished [58]. Lower OLR is recommended for high TSS removal efficiency, as it aids stabilization of the accumulated solid substrate ingredients, particularly in low ambient temperature conditions [58]. Prasanth et al. [79] showed that presence of COD_P in synthetic wastewater minimizes the biodegradability rate constant, substrate biodegradability, anaerobic degradability, substrate activity, and sludge activity.

Table 1. Selected key operating parameters and performance of laboratory-scale (reactor size range 2–35 L) UASB systems studied by different researchers.

Inocula	Influent Characteristics		OLR (kg COD/m^3 day)	HRT (h)	Temperature (°C)	% Removed		Methane (CH_4) Collection (L/g COD Removed)	Remarks	References
	COD_T	COD_S				COD_T	SS			
Digested sludge	350–500	150–300	—	48–10	20	60–75	86	0.21–0.26	• Diluted municipal wastewater was used. • Sulphate reduction: 70 to 80%. • Lower CH_4 recovery (30 to 40%) as 30–40% of the COD_T was consumed in sulphate reduction.	[80]
Inoculum mix obtained from anaerobic digesters treating primary and activated sludge, fish canning and sugar wastewaters.	693	322	Increased to 3	24–5	—	85 (at 24h HRT) 53 (at 5h HRT)	89 (at 24h HRT) 63 (at 5h HRT)	—	• CH_4 recovered in the biogas ranged from 25% to 30% of the influent COD, increasing slightly with the operational time.	[81]
Granular Sludge	312 ± 73.2	114 ± 30.4	1.6	4.7	13–25	69.4	—	0.16–0.26	• An amount of 390 g mixed liquor suspended solids (MLSS) with 91% of volatile matter was seeded to the reactor.	[78]
Granular sludge	456 ± 129	112 ± 34	—	8	13	67 ± 18	—	0.25 ± 0.04	• Treatment of raw sewage with small sludge granules under low up-flow velocity and low temperature was not practical due to the sludge bed flotation. • Conversion of removed COD to CH_4 = 72 ± 12%.	[61]
Anaerobically digested municipal sludge	310	—	—	12	15	48	44	—	• Low biogas production could not serve for an effective mixing of sludge. Mechanical stirrer installed in the reactor for sludge-wastewater contact did not lead to an improvement in removal efficiency.	[82]
	310	—	—	12	9	37	38	—		
Granular sludge	600 ± 50	170 ± 40	1–2	4.8–10	33	84	—	0.29	• Synthetic wastewater was used. • CH_4 content of the biogas = ~ 96%.	[25]
Flocculent sludge from municipal anaerobic digester	700–1000	50–70% of COD_T	—	15 10 8 6 4	25 ± 1	76 ± 10 79 ± 4 83 ± 7 92 ± 5 89 ± 4	—	—	• Reactor was inoculated with 13.5 g VSS/L of flocculent or granular sludge. • Synthetic domestic wastewater (made from a mixture of dog food, clay, sucrose, and peptone) was used. • The composition of these materials was chosen to maintain the ratio of organic constituents in the sewage (protein 50%, carbohydrates 40%, fat 10%).	[60]
Granular sludge from UASBR treating brewery wastewater			—	15 10 8 6 4		81 ± 11 84 ± 14 86 ± 8 91 ± 4 87 ± 3	—	—		
Granular sludge treating alcohol distillery wastewater	165–270	—	0.7	7.5	13 ± 2	24–54	—	—	• Raw sewage used in this study presented a weak character, which was due to seawater addition into the sewerage system to prevent purification and for cleaning the pipes during the sampling period in summer.	[83]

Note: (1) "—" = Information not provided. (2) All values are in mg/L unless otherwise mentioned.

Table 2. Selected key operating parameters and performance of laboratory-scale (reactor size range 55–6000 L) UASB systems studied by different researchers.

Inocula	Influent Characteristics		OLR (kg-COD/m^3 day)	HRT (h)	Temperature (°C)	% Removed		Methane (CH_4) Collection (L/g COD Removed)	Remarks	References
	COD_T	COD_S				COD_T	SS			
Sugar beet cultivated sludge	117–1253	69–666	—	—	9–19.5	65–89	—	0.085–0.32	• Specific activity of the seed sludge: 0.8–1.0 kg COD/kgVSS/day at 30 °C. • Under dry weather conditions, a higher COD reduction was achieved compared to wet weather conditions for a given temperature and hydraulic loading.	[84]
Granular Sludge	100–900	—	—	9–16	10–18	46–60	—	—	• Reactor volume: 6000 L.	[85]
Flocculent Sludge	406–424	—	—	4	20–23	60–65	69	0.10–0.12	• Removal efficiencies obtained in winter and summer periods were about the same. The average ambient temperature was 17 °C in the winter period and 22 °C in the summer period, the average temperature inside the reactor being 20 °C in winter and 23 °C in summer.	[70]
Digested sludge from an anaerobic digester treating sewage	500 (For 200 day) 300 (For 60 day)	—	3 4 2 1.2	4 3 6 6	20–35	83–88	—	0.141	• BOD reductions = 90–92%. • Due to the presence of a high concentration of active granular sludge in the lower portion of the reactor, an 80% reduction in COD occurred within the bed level itself.	[86]
Flocculent sludge from a pilot scale UASBR treating domestic sewage. Digested primary sludge from a wastewater treatment plant.	721 (UASBR) 460 (UASB-digester system)	—	2.88 ± 0.69 (one-stage UASBR) 1.84 ± 0.49 (two-stage UASBR)	6 (UASBR) 21.2 ± 1.5 days (CSTR)	15 (UASBR) 35 (CSTR)	44 ± 9 (UASBR) 66 ± 6 (UASBR)	—	—	• The sludge re-circulation in the UASBR improved both the physical removal of solids and the conversion as it increased the methanogenesis from 20% in the one-stage UASB to 47% in the UASBR. • Excess sludge production from the UASBR was dewaterable and also well stabilized.	[4]
Anaerobic sludge discharged from a UASBR	816 555 298 195 92 770 787 716	566 420 216 120 55 450 512 486	3.3 2.2 1.2 0.8 0.4 4.6 9.4 17.6	6 4 2 1	27 ± 1	57 60.1 64 53.3 50.4 45.5 44.1 36.6	— — — — — — — —	0.472 0.347 0.201 0.107 0.48 0.351 0.312 —	• Maximum COD removal was achieved with an HRT longer than 4 h, and influent COD concentration higher than 300 mg/L.	[87]
Without inoculum	1159–1701	548–1176	3.35 ± 0.32	10	Designated as hot period	43–69	27.8 ± 3	—	• The low temperature during winter and the high strength and solids content limited the performance of the investigated one-stage UASB.	[88]
	770–1525	875–1244	2.73 ± 0.45		Designated as cold period	5–57	—	—		
Sludge from a pilot scale UASBR	1465 ± 60	783 ± 100	2.7 (UASBR) 3.7 (AF)	15 (UASBR) 4 (AF)	23.5 ± 0.5	32 (UASBR) 35 (AF) 55 (Total System)	—	—	• A two-stage UASB-AF system was studied, which allowed for a total volume reduction of 17% compared to a single stage anaerobic reactor. • Start-up period = 7 months. • Discharged sludge from the AF was recycled back to the UASBR.	[89]

Note: (1) "—" = Information not provided. (2) All values are in mg/L unless otherwise mentioned.

Table 3. Selected key operating parameters and performance of pilot to full-scale (reactor size range 20–30,000 m^3) UASB systems studied by different researchers.

Inocula	Influent Characteristics		OLR (kg-COD/m^3 day)	HRT (h)	Temperature (°C)	% Removed		Methane (CH_4) Collection (L/g COD Removed)	Remarks	References
	COD_T	COD_S				COD_T	SS			
Digested cow manure	267	95	—	6-8	25	75–82	70–80	—	• VSS/TSS ratio: 50. • Start-up period: 6 months.	[90]
—	400 ± 64	171 ± 34	—	14.5	19 ± 3	64	—	0.09	• BOD removal: 60–72%. • COD_S removal: 74–84%. • Presence of sulphate and sulphide in wastewater caused sulphate-reducing bacteria to inhibit the activity of the methanogenic bacteria competing for the same substrate (acetate and hydrogen).	[91]
	403 ± 68	156 ± 37		11	18 ± 3	63	75	0.09		
	407 ± 61	151 ± 20		8.8	21 ± 3	65	75	0.11		
	459 ± 84	160 ± 17		7.2	22 ± 3	55	66	0.12		
	374 ± 31	139 ± 10		7.0	22 ± 2	59	76	0.12		
	194 ± 65	96 ± 31		7.5	25 ± 2	54	62	0.25		
	188 ± 37	96 ± 21		6.1	28 ± 1	56	64	0.15		
	258 ± 50	115 ± 29		6.2	25 ± 2	60	67	0.15		
	307 ± 63	120 ± 28		5.1	21 ± 3	62	67	0.13		
Without inoculum	563	—	—	6	20–30	74	75	0.05–0.10	• Start-up of the UASBR was successfully achieved within 10 weeks. • Produced excess sludge had excellent dewatering and drying characteristics and was well stabilized. • During winter, the treatment efficiency and process stability remained good.	[72]
Granular sludge grown on paper mill wastewater from full scale UASB	391	164	—	2–7	≥13	16–34	—	—	• 54 L of seed material was used with standard acetotrophic methanogenic activity 0.17 g CH_4-COD/g VSS.d at 30°C.	[57]
Without inoculum	380	—	2.0 (maximum)	5–19	—	66–72	—	—	• UASB provided a cost-effective and efficient solution in treatment of sewage in tropical climates.	[71]
Digested sludge added 10% (V/V)	436	402	—	7	16 (Winter average) 23 (Summer average)	74	87	—	• Total BOD removal: 80%. • The plant operated at a low inlet flow, which is only 17% of the design flow. • No requirement of any excess sludge discharge.	[92]
—	500		2.0	6	20	75	—	—	• A homogenization tank was used as a pre-treatment unit before UASB treatment. • Slow filtration was used as a post-treatment before reusing the water for watering purpose.	[93]
—	300	—	1.2	6	20	70	—	—	• Pre-treatment units before UASB: screen, grit chamber, and grease inceptor. • Post-treatment unit after UASB: secondary settler, chlorination.	[93]
—	500	—	1	12	20–25	70–80	—	—	• Pre-treatment units before UASB: screening. • Post-treatment unit after UASB: Aerobic submerged filter, secondary settler, and chlorination. • Biogas production: 23–25 m^3/day.	[93]

Table 3. *Cont.*

Inocula	Influent Characteristics		OLR (kg-COD/m^3 day)	HRT (h)	Temperature (°C)	% Removed		Methane (CH_4) Collection (L/g COD Removed)	Remarks	References
	COD_T	COD_S				COD_T	SS			
Without inoculum	1419–1650	—	3.6–5.0 & 2.9–4.6 kg COD/m^3d for stage I and stage II, respectively (during 1st year). Only stage I was operated as a single-stage UASBR at half of the previous loading rate (during 2nd yr).	8–10 (I stage) 5–6 (II stage)	18–25	62 (summer) 51 (winter)	60 (summer) 55 (winter)	0.439 0.249	• Single stage UASBR operated at OLR of 1.5–1.8 kg COD/m^3 day (average HRT = 24 h) resulted in 87–93% COD removal. • CH_4 content was 75% and 65% during summer and winter, respectively.	[94]
—	375 ± 97.6 403 ± 66.2 390 ± 64 443 ± 101.9 318 ± 101.9	246 ± 50 179 ± 62 157 ± 54 213 ± 58 213 ± 58	—	9.9 9.6 9.8 10.3 9.4	27 ± 6 28 ± 6 29 ± 6 29 ± 6 24 ± 6	~46.7 ~45.4 ~38.5 ~41.3 ~62.3	~44.4 ~42.5 ~35.7 ~48.4 ~34.0	—	• Performance of five full-scale UASB-based STP were performed with the reactors size ranging from 11,200–30,000 m^3.	[95]
—	440	—	—	8	—	60	49	—	• Plant capacity 164,000 m^3/day. • reactors each with a volume of 2286 m^3. • Up-flow velocity 0.57 m/h.	[75]
	549			8		67	70		• Plant capacity 90,000 m^3/day. • reactors each with a volume of 2705 m^3. • Up-flow velocity 0.44 m/h.	
	544			—		58	53		• Plant capacity 48,000 m^3/day.	
	519			7.5		49	50		• Plant capacity 38,000 m^3/day. • reactors each with a volume of 2029 m^3. • Up-flow velocity 0.60 m/h.	
	1293			—		77	56		• Plant capacity 30,000 m^3/day.	
	602			7.8		44	45		• Plant capacity 120,000 m^3/day. • reactors each with a volume of 1960 m^3. • Up-flow velocity 0.62 m/h.	
	459			7.7		49	51		• Plant capacity 43,000 m^3/day. • reactors each with a volume of 2304 m^3. • Up-flow velocity 0.62 m/h.	
	697			10.3		52	84		• Plant capacity 49,000 m^3/day. • reactors each with a volume of 2645 m^3. • Up-flow velocity 0.48 m/h.	

Note: (1) "—" = Information not provided. (2) All values are in mg/L unless otherwise mentioned.

Therefore, several researchers have investigated the feasibility of installing a hydrolysis unit prior to UASB to reduce the loading of COD_P on the reactor. The following section includes a review of studies carried out on a two-phase UASB with pre-hydrolysis units.

3.2. Sewage Treatment in a Two-Phase UASBR with Hydrolysis

Van Haandel and Lettinga [63] proposed the two-phase anaerobic treatment of sewage, which involves the separation of the non-methanogenic and the methanogenic digestion phases into separate reactors [96–98]. The first step of a two-phase UASB treatment system is mainly aimed at the removal of suspended solids and partial hydrolysis and acidification. Its effluent is subsequently treated in a second step methanogenic UASBR, which is devoted to the removal of soluble organic matter [32,99]. The removal efficiency of suspended solids in the hydrolysis reactor is higher than that of organic matter and excess sludge is to be discharged regularly. As a result, the sludge age remains relatively low in this reactor, hindering the development of the slow-growing methanogens, reducing methanogenesis to a minimum. Moreover, the development of acid fermentation may tend to reduce the pH to a value below the optimal range for methanogenic microorganisms [5]. The effluent from the first reactor predominantly contains organic matter in a dissolved state.

Methanogenesis governs the kinetics of anaerobic treatment of soluble wastewater. However, hydrolysis of solids has been reported to be the rate-limiting step for the treatment of wastewater containing COD_P [79]. The concept of separating non-methanogenic to methanogenic digestion steps needs to be shifted to first phase hydrolysis and subsequent anaerobic biotransformation in a UASBR. The separation between non-methanogenic and methanogenic phases has been applied to the treatment of soluble wastewater. The term soluble wastewater refers to the municipal wastewater or sewage that has majority of its total COD as soluble COD (COD_S). In such a case, the first phase is fast and the second, methanogenesis, is slow. For the treatment of complex sewage, containing cellulose, soluble starch, and glucose, hydrolysis of cellulose has been reported to be the rate-limiting step in the overall anaerobic digestion process [100]. Considering this, hydrolysis should, in principle, be carried out at a higher HRT/SRT ratio than methanogenesis. In addition to this, UASBRs perform better at higher OLRs. It is necessary to curb the methanogenesis in the hydrolysis reactor. In the first phase of the two-phase hydrolysis–UASB systems, hydrolysis should be maximized and methanogenesis should be minimized.

Pretreatment of complex wastewaters is often required to (i) lower the elevated percentage of particulates, (ii) increase the biodegradability of the remaining COD, (iii) favor the subsequent biological elimination of nutrients, (iv) stabilize the sludge, totally or partially; and (v) reduce the bulking in the activated sludge process. Pretreatment processes have achieved reductions of 63–83% of suspended solids and 25–43% total COD (COD_T) [32,101,102]. There are several pretreatment options, namely completely stirred tank (CSTR) reactor [76,103], UASR reactor [33,99] and HUSB reactor [32,104,105]. The type of pretreatment depends on the nature of complexity in the wastewater. Hydrolysis is generally a preferred option for a wastewater containing COD_P as a complexity. The CSTR pretreatment option involved inclusion of a CSTR acid-phase digester before an up-flow methane-phase digester [87]. To provide optimal contact conditions and recycle liberated indigenous enzymes or cell biomass fractions, continuous recycling of the effluent sludge to the sludge bed of the reactor was used. The two-phase digestion process performs better than conventional one-phase up-flow anaerobic digester. The TVS reduction of the two-phase system was 53%, which was higher than the one-phase system [87]. The pretreatment of complex wastewater containing a high fraction of suspended solids in a UASR reactor involves a two-phase system with a high loaded UASB reactor as first stage [83]. The applied high loading rate in the first stage reactor will result in little if any gas production, and therefore a high suspended solids removal was achieved [83]. The HUSB reactor is another pretreatment option where removal of SS is carried out resulting in accomplishing a certain sludge stabilization and raising the biodegradability of the remaining COD of the sewage [49].

Among the main components of primary sludge (e.g., carbohydrates, lipids and proteins), carbohydrates are known to be easily and rapidly converted to simple sugars via hydrolysis and subsequently fermented to volatile fatty acids (VFA). Protein is hydrolyzed to amino acids and further degraded to VFA either through anaerobic oxidation linked to hydrogen production or via fermentation. The former is dependent on the presence of hydrogen-scavengers while the latter is independent of the methanogenic activity in the reactor. Among the lipids, triglycerides are hydrolyzed to long chain fatty acids and further oxidized via β-oxidation to acetate or propionate [106]. In a primary fermentation unit, COD removal of about 25% was observed in almost all cases, even though with HRTs of 2.8 and 3.3 h, suspended solid removal was about 70% [101]. This imbalance between suspended solid and COD removal is due to the solubilization of a segment of the particulate organic matter in the wastewater. The reduction in COD, due to the lowering of COD_P concentration, is offset by an increase in soluble COD (COD_S) concentrations. No production of dissolved COD was observed with an HRT of 1.1 h and it increased gradually with an HRT between 2.1 and 2.8 h. A maximum produced dissolved COD of around 30 mg/L was observed for HRT between 2.1 and 2.8 h. At HRT > 2.8 h, the concentration of the filtered COD decreased, due to the action of methanogenic microorganisms. At a HRT of 4.3 h, dissolved COD concentration was on the order of 19 mg/L [101].

The hydrolysis of lipids and carbohydrates increases with increasing SRT, whereas protein hydrolysis only occurs under methanogenic conditions [106]. This study employed five completely mixed stirred tank reactors (CSTRs) with an effective volume of 5 L and operated them to maintain SRTs of 3, 5, 8, 10, and 15 days. The process temperature was controlled at 25 ± 1 °C by recirculation of temperature-controlled water through the double walls of the reactors. The reactors were inoculated with diluted digested primary sludge (20 gTS/L), i.e., settled solids of domestic sewage from a wastewater treatment plant. The feed included diluted primary sludge from the same wastewater treatment plant. A certain volume of digested sludge was withdrawn from the reactor and an equal volume of primary sludge was pumped into each reactor. The reactors operated at 3 and 5 d SRTs were fed two times a day to avoid shock loading [91]. The study revealed a decrease in protein hydrolysis and acidification under acidogenic conditions. The low values obtained for protein hydrolysis and acidification have been partially explained by the relatively high ammonium-nitrogen concentration in the influent, which suggests that easy degradable protein was already hydrolyzed before the sludge was used in the CSTRs. Also, low pH and high lipid concentrations could affect the hydrolysis and precipitation of ammonium as struvite may contribute to the decrease of the ammonium-nitrogen levels, which might have contributed to a lower calculated hydrolysis [91]. Also, the hydrolysis rate of entrapped organics has been reported to be significantly affected by temperature, that is, 58% of entrapped particulate organics liquefy at 25 °C, which decreases to 33% at 13 °C [78]. Maximum solubilization occurs only when CH_4 production is effectively suppressed. Little, if any, methanogenesis will develop in the hydrolytic reactor, because the pH is depressed by acid fermentation. Only a part of the entrapped matter will be hydrolyzed and excess sludge will have to be discharged from the reactor at a relatively high frequency. This means that the sludge age will be too low for the slow growing methanogens. The effluent from the hydrolytic reactor will be mainly dissolved compounds, so that it can be conveniently treated in second phase reactor [63]. The results of pre-hydrolysis of domestic wastewater in bench and pilot-scale solubilization reactors under different working conditions are presented in Table 4. Studies on the hydrolysis of particulates in sewage under anaerobic conditions have, so far, been carried out on bench scale reactors [76]. In all the cases, hydrolysis is accompanied by reduction in COD (Table 4). The range of COD reductions is about 25–59%. The reduction of COD_S, in most cases, indicates a conversion of soluble fractions into CH_4 gas. This indicates that methanogenesis has taken place, in addition to hydrolysis. The reasons for methanogenesis in hydrolysis reactor or pretreatment unit may be due to good amount of seed sludge or a low food to microorganism ratio (F/M < 1) and other microenvironment parameters. Efforts are required to check methanogenesis in hydrolysis unit.

Table 4. Hydrolysis performance on bench-scale and pilot-scale solubilization reactors for the treatment of domestic wastewater.

Treatment Used before Methane Fermenter	Type of wastewater (Temperature, °C)	HRT, h (OLR, g COD/L-day)	Performance of Hydrolysis Reactor					COD_s (Effluent)/COD_s (Influent)	Remarks	Reference
			Influent COD_T mg/L (% Removal)	Influent COD_S mg/L (% Removal)	Influent SS mg/L (% Removal)	Influent VSS mg/L (% Removal)	Influent VFA mg/L (Effluent VFA mg/L)			
HUSB (37 L)	Domestic Sewage (17)	3 (—)	697 (38%)	197 (−2.6%)	237 (83%)	— (—)	59* (107*)	1.026	- Low hydrolysis and acidification rates at lower temperature resulted in production of less soluble matter as compared to higher temperature.	[32]
	Domestic Sewage[a] (11)		318 (11%)	100 (7.3%)	171 (77%)	— (—)	13 (34*)	0.927		
	Domestic Sewage (12)		507 (37%)	116 (16.1%)	154 (75%)	— (—)	40* (73*)	0.839		
Up-flow Sludge Blanket Fermenter (0.79 m^3)	Domestic Sewage (20 ± 1)	1.1–4.3 (—)	462.3 (27.5%)	213 (–7.04%)	167.3 (60.4%)	— (—)	44 (88)	1.070	- Maximum solubilisation occurred at HRT of 3.3 h	[101]
UASR (7.5 L)	Domestic Sewage (14–21)	3 (5.6)	697 (38.0%)	138 (31.2%)	— (—)	— (—)	— (—)	0.704	- Pretreatment of raw sewage was mainly through removal of suspended solids and acidification of readily available dissolved COD	[99]
CSTR (5 L)	Domestic Sewage (25)	— (—)	30851 ± 210 (—)	— (—)	— (—)	— (—)	— (—)	— (—)	- Hydrolysis of lipids and carbohydrates increased with increasing SRT, whereas protein hydrolysis only occurred under methanogenic conditions. - SRT < 8 day resulted in acidogenic conditions, - SRT > 8 day resulted in methanogenic conditions	[106]
Hydrolytic Up-flow Digester (2 L)	Urban Wastewater (20)	2.2–4.5 (4.42)	525–710 (33%–47%)	257–344 (13%–30%)	186–268 (55%–68%)	153–232 (52%–63%)	6–29 (25–107)	0.69–0.89	- The methanogenic activity of the inoculum used was 0.18 g COD_{CH4}/g VSS·d at 20 °C	[107]
HUSB (485 mL)	Urban Wastewater (20)	2.2–26.7 (0.9–7.3)	645 (33%)	302 (14.2%)	239 (60.7%)	197 (57.4%)	21 (101)	0.86	- Sludge granulation was observed after 150-day of operation, at an HRT of 3.4 h - Granules had a weak structure and low density, with the specific methanogenic activity of the sludge being about 0.24 g CH_4 - COD/gVSS·day. - COD_T and CODs removal of 57% and 76%, respectively at an HRT of 3.4 h and an OLR of 5.6 kgCOD/m^3·day.	[108]
Prefermenter Reactor (3.3 L)	Domestic Sewage (17–20.5)	1.3 (7.36)	399 (25%)	126 (9.5%)	305 (61%)	—	5.1 (14)	0.90	- SRT of 5 day and 10 day were tested and the best results were obtained in a covered pre-fermenter with a 5 d SRT.	[109]
AF (60 L)	Domestic Sewage (13 °C)	2–4 (—)	425–533 (58.6–70.6)	130–172 (53.6–55.2)	—	—	33–55 (77.9–97)	0.32–0.36	- The AF reactor represented an efficient pre-treatment process for domestic sewage at 13 °C. - Average COD_{SS} removal efficiencies amounted to 81%, 57% and 58% at an HRT of 4, 3 and 2 h, respectively.	[54]

Table 4. *Cont.*

Treatment Used before Methane Fermenter	Type of wastewater (Temperature, °C)	HRT, h (OLR, g COD/L-day)	Performance of Hydrolysis Reactor					COD_s (Effluent)/COD_s (Influent)	Remarks	Reference
			Influent COD_T mg/L (% Removal)	Influent COD_S mg/L (% Removal)	Influent SS mg/L (% Removal)	Influent VSS mg/L (% Removal)	Influent VFA mg/L (Effluent VFA mg/L)			
CSTR	Synthetic Wastewater (30 ± 2)	1–6 (2.32–13.96)	582 (38.3)	314 (7.96)	229 (52)	183.5 (50)	0 (69)	0.45	- Synthetic wastewater was used in this study replicate municipal wastewater	[76]
HUSB	Domestic Wastewater (17.5–20.5)	2.9–7.1 (1.22–3.88)	361–469 (45.8~58.9%)	115–121 (−28.9~−52.5%)	188–373 (81.7~84.9%)	169–283 (78.7~85.2%)	6–9 (68–87)	1.289–1.525	- SRT in the system varied from 10.4–50.5 days. - Biogas with a CH_4 content of 55 to 70% was recovered but with low CH_4 generation rate.	[105]
HUSB (0.69 m^3)	Municipal Wastewater (—)	3–7 (—)	699–739 (33–51%)	—	568–634 (76–89%)	—	—	—	- OLR = 0.5–4 gBOD/L-day - Average BOD_5 removal was 48%.	[104]

3.3. Kinetics of Anaerobic Digestion

In anaerobic digestion, the rate-limiting step of the overall process is related to the nature of the substrate, process configuration, temperature, and loading rate [110]. Hydrolysis, a pretreatment process, is used to degrade complex polymeric materials such as polysaccharides, proteins, and lipids (fat and grease) to simple soluble products by extracellular enzymes, secreted by microorganisms, so as to facilitate their transport or diffusion across the cell membrane [111]. Aerobic, anaerobic and facultative microorganisms can catalyze hydrolysis of polymers. Biopolymers are mostly insoluble, except for some small protein molecules, dextran, and they form fibers (cellulose), grains (starch) or globules (casein after enzymatic precipitation) or can be melted or emulsified (fat). While aerobes oxidize acetate in the tricarboxylic acid cycle and respire the reducing equivalents as oxygen, the anaerobes (e.g., Ruminococcus sp., Clostridium sp., or Eubacterium sp.) either releases molecular hydrogen or transforms pyruvate or acetate to highly reduced metabolites, such as lactate, succinate, ethanol, propionate, or n-butyrate. These reduced metabolites are further oxidized within the anaerobic food chain anaerobically by acetogenic microorganisms [41].

Both freely soluble exo-enzymes, diluted in bulk mass of liquid and enzymes excreted by the neighboring microorganism colonies growing on the surface of the particles, catalyze the hydrolysis process. The ratio of surface area to particle size of the sludge has been reported as an important aspect for the hydrolysis of particulate organic matter. In the case of glucose, starch, carboxymethyl cellulose, casein, and food residues from a restaurant, hydrolysis proceeded faster than methanogenesis, whereas hydrolysis was the rate-limiting step for newspapers and leaves [41]. Cellulose and lignin are the most abundant biopolymers.

Cellulose fibers are implanted in a matrix of hemicelluloses, pectin, or lignin. To make cellulose fibers available to microorganisms, the hemicellulose, pectin, or lignin matrix must be degraded either by microbial action or chemical solubilization. The enzyme glycosyl hydrolases are involved in the degradation of cellulose and hemicellulose by cleaving the glycosidic bonds between different carbohydrates and between carbohydrates and non-carbohydrates. Cellulosomes, stable enzyme complexes formed in microorganisms, are active in degrading crystalline cellulose. Hydrolysis of biological structural components such as cellulose and lignin polymers is difficult. In comparison with the slow hydrolysis of celluloses, starch can be easily hydrolyzed, which is mainly facilitated by the branching, helical structure of starch. While the cellulose forms fibers with a large surface covered with lignin, the starch forms grains with an unfavorable surface-to-volume ratio for enzymatic cleavage. Thus, the hydrolysis rate is limited by inadequate access of the enzymes to the substrate. Whereas cellulose and starch are biodegradable, other carbohydrate-derived cellular compounds are not biodegradable and—after reaction with proteins—form humic acid-like residues [41].

Oxidation of dead biomass proceeds anoxically or anaerobically through the reduction of electron acceptors such as nitrate and nitrite or the reduction of sulfate, Fe^{3+}, Mn^{4+}, or CO_2, respectively. Biopolymers of leaves or the plants decompose by extracellular enzymatic hydrolysis. The monomers are fermented, and the fermentation products may be degraded further to biogas by acetogenic and methanogenic microorganisms. Single cultures of strictly anaerobic microorganisms are not capable of complete degradation of biopolymers to CH_4 and CO_2. Under anaerobic conditions, biopolymers must be degraded by a food chain via depolymerization (hydrolysis), fermentation (acidogenesis), oxidation of fatty acids (acetogenesis), and biogas formation (methanogenesis) as the last step [112]. Henze et al. (1997) reported values of hydrolysis constants k_h for dissolved organic polymers to be 3–20 day^{-1} under aerobic conditions and 2–20 day^{-1} under anaerobic conditions [113]. The values of the hydrolysis constant k_h for particulate solids were reported to be 0.6–1.4 day^{-1} under aerobic conditions and 0.3–0.7 day^{-1} under anaerobic conditions [113].

The kinetic description of anaerobic degradation of complex organic matter has generally been accomplished through the rate-limiting hydrolysis step approach. Monod, zero and first-order kinetic models have been used to represent the biodegradation of domestic sewage in a combined treatment system. Of these, the first-order model is the only one that adequately represents biodegradation

in both aerobic and anaerobic parts of the system. In the anaerobic unit, the first order kinetic constant is 0.31 h^{-1} in summer temperatures (~19 °C) and 0.20 h^{-1} in winter temperatures (~12.5 °C), whereas in aerobic units, the values are higher (~2.0 h^{-1}) [114]. Table 5 gives the characteristic values of maximum growth rate constants of biomass and first-order hydrolysis constants used in METHANE model [115,116]. The hydrolysis step of complex organic matter has been identified as the rate-limiting in anaerobic digestion [115,117]. Acetogenesis or methanogenesis might be the rate-limiting stages in complex waste. For complex waste, stimulation of hydrolysis (mechanically, chemically or biologically) could lead to a further inhibition of acetogenesis or methanogenesis stages (these stages could be rate limiting for complex waste), which ultimately affects hydrolysis as well [117].

Table 5. The characteristic values of maximum growth rate constants (day 1) of biomass and first-order hydrolysis constants used in METHANE model. [115].

Feed, Temperature	Process				Source of Experimental Data
	Hydrolysis	Acidogenesis	Acetogenesis	Methanogenesis	
Cellulose, 35 °C	0.1	5.6 (B1), 4.1 (B2)	0.56	0.56 (H)	[100]
Sewage Sludge, 5 °C	0.25	5.0 (B1), 5.0 (B2)	0.8	0.5 (A), 2.0 (H)	[118]

Note: B1 = acetate-producing acidogens, B2 = propionate-producing acidogens, A = acetate-utilizing methanogens, H = hydrogen-utilizing methanogens.

The International Water Association (IWA) developed a structured generic model for anaerobic digestion titled "Anaerobic Digestion Model No 1 (ADM1)", which included multiple steps to describe the biochemical as well as physico-chemical processes during anaerobic digestion [119]. The biochemical processes were divided into: (a) disintegration of dead biomass to particulate constituents, (b) enzymatic extracellular hydrolysis of these particulates to their soluble monomers, (c) intercellular degradation of soluble materials (resulting in biomass growth and subsequent decay) [119]. Meanwhile, the physico-chemical processes were not biologically mediated and included ion association/dissociation, and gas-liquid transfer [119]. This model assumed a disintegration step of complex particulate waste to carbohydrate, protein, and lipid particulate substrate and particulate and soluble inert material to facilitate modeling of waste-activated sludge digestion. The disintegration step was included to precede more complex hydrolytic steps and this step makes the complex polymeric material more bioavailable for subsequent hydrolysis. This disintegration step includes an array of processes such as lysis, non-enzymatic decay, phase separation, and physical breakdown (e.g., shearing). ADM1 is a powerful tool for predicting steady-state behavior of anaerobic digesters treating sewage sludges [120]. Determination of the fraction of the biodegradable versus the total solids in the feed sludge is critical while using the ADM1 model. A first-order disintegration process was employed to describe the complex composite particulate waste breakdown to particulate substrates of carbohydrate, protein, and lipid along with inert materials. When the disintegration step was considered, before the hydrolytic steps in ADM1, it was found to be the rate-limiting step compared to the hydrolysis step and hence it affected the final modeling results.

The use of the ADM1, with the first-order rate constant assigned for disintegration (K_{dis}) and hydrolysis (K_{hyd}) as 0.5 day^{-1} and 10 day^{-1}, respectively, reflected the steady-state full-scale anaerobic digestion data collected from two different sized wastewater treatment plants with satisfactory level of accuracy [120]. The ADM1 is used extensively for modeling anaerobic digestion of solid wastes and has been studied and modified to reflect different physico-chemical and biochemical processes in the anaerobic digestion process [121–124]. The ADM1 models the disintegration and hydrolysis steps in anaerobic digestion following a first-order kinetics and assuming that their rates do not depend on disintegration/hydrolytic biomass concentration. However, for complex substrates, the first-order kinetics can be modified to account for slowly degradable material [117,125]. It has been demonstrated that when hydrolysis is coupled with the growth of hydrolytic microorganisms, then the model prediction is enhanced at high or fluctuant organic loading [117]. Ramirez et al. [125]

developed a slightly modified ADM1 model to simulate thermophilic anaerobic digestion of thermally pretreated waste activated sludge by using the Contois model for disintegration and hydrolysis steps instead of first-order kinetics and the Hill function to model for ammonia inhibition of aceticlastic methanogens instead of a non-competitive function.

The modified ADM1 was calibrated and validated using batch experimental data sets and the model parameters involve three disintegration biochemical parameters, nine hydrolytic biochemical parameters and four stoichiometric parameter values. The model was capable of predicting the data measured under different pretreatment conditions and was able to explain the dynamics of acetate accumulation in batch experiments [125]. ADM1 was also calibrated and validated (using 360 days operation data) to satisfactorily model the dynamic performance of a full-scale anaerobic digester treating mixed (primary and secondary) sludge in a wastewater treatment plant under mesophilic condition [126]. The calibration of the ADM1 parameters requires a deep understanding of the interaction between each process, functional microorganism and environmental conditions. Thus, only the most important parameters should be calibrated to improve the compatibility between measured data and model outputs. A minimum set of parameters (disintegration rate of particulates, fractionation of particulate organics and kinetics of acetate, propionate and hydrogen utilizers), which were reported as the most sensitive parameters in the simulation results, were manually calibrated [126].

4. Comparison of UASBRs with and without Pre-Hydrolysis Units

The following sections provide comparisons between treatment efficiency of the single-stage UASB and two-phase hydrolysis-UASB systems in terms of solids reduction, removal of various COD fractions, and sludge production.

4.1. Suspended Solids Reduction

One of the main purposes of adopting a two-phase hydrolysis-UASB system is to remove and digest the suspended solids in the hydrolysis step, which might otherwise accumulate and reduce methanogenic activity in the anaerobic digestion process. Accumulation of solids might also lead to increase in sludge bed height, resulting in heavy wash-out of sludge particles [58]. From Table 3, it can be observed that the one-stage UASB process was able to remove 34–84% of suspended solids from domestic wastewater. On the other hand, the hydrolysis unit of the two-phase process was able to remove 52–89% of suspended solids (Table 4). Studies observing overall performance of a two-phase system reported an overall removal efficiency of suspended solids of 76–93% (Table 6). Lin and Ouyang (1993) [103] demonstrated that the ratio of total volatile solids and total solids (TVS:TS) in the two-phase system was lower than that of the one-stage system. The TVS reduction was 43–53% in the two-phase system, which was higher than that of the one-stage system [103].

Table 6. Overall domestic sewage treatment performances in two-phase hydrolysis-UASB processes.

System Configuration (Hydrolysis Unit → Anaerobic Digestion Unit)	Temperature, °C	OLR in First Phase and Second Phase, kg COD/m^3-day	HRT, h (First Phase + Second Phase, h)	Influent COD_T, mg/L (% Removal)	Influent COD_S, mg/L (% Removal)	Influent SS, mg/L (% Removal)	References
HUSB → UASB	12,17	— (first phase) 2.4–5.0 (second phase)	5 (3+2)	318–697 (51–71%)	100–197 (41–51%)	154–237 (76–83%)	[32]
UASB → UASB	18–20	1.22–2.75 (first phase) 1.70–6.20 (second phase)	10~6 (8~4+2)	200–700 (74–82%)	45–55% of COD_T (73–100%)	90–385 (86–93%)	[127]
HUSB → UASB	17	5.3 (first phase) 4.0 (second phase)	5 (3+2)	650 (69%)	187 (79%)	217 (83%)	[128]
AF → AH	13	—	6–12 (2+4, 3+6, 4+8)	425–533 (58.6–70.6%)	130–172 (53.6–55.2%)	— (—)	[54]

4.2. Removal of COD

It has been reported that average COD_T removal efficiency for one-stage and two-phase UASB systems is 74% and 80%, respectively [5]. Table 3 indicates COD_T removal efficiencies between 51 and 82%

in one-stage UASB systems. On the other hand, the hydrolysis stage of the two-phase process indicated COD_T removal efficiencies of 11–59% (Table 4). Table 6 demonstrates the overall COD_T and COD_S removal performance of a two-phase system. The overall COD_T removal efficiency of a two-phase process has been reported to be between 51–82%. It is to be noted that COD_T removal efficiency depends on issues like temperature and HRT. For similar temperatures, the two-phase process exhibited better COD removal than the one-stage UASB process [32]. Removal efficiency of COD_T increases considerably with the decrease of the HRT, as a treatment efficiency of 76% was achieved at HRT = 10 h (8 h for Phase I and 2 h for Phase II), while the treatment efficiency was 82% at HRT = 6 h (4 h for Phase I and 2 h for Phase II) [127]. The two-step AF→AH system, operated at an HRT of 4 + 8 h and a temperature of 13 °C, provided high removal efficiencies for all fractions of COD. The COD_T removal efficiency was as high as 71%, similar to that in one-step UASBRs in tropical countries. Application of the AF→AH system results in high values for hydrolysis, acidification, and methanogenesis. No significant difference was observed at different HRTs. At the imposed HRTs of 2 + 4 to 4 + 8 hours in the AF→AH system, 60–74% of the removed COD_T was converted to CH_4 [54]. These ranges are significantly higher than those reported by Uemura and Harada (2000) [78], who observed that 35% of the removed COD_T was converted to CH_4 while treating domestic sewage in a one-stage UASBR (at HRT = 4.7 h and temperature = 13 °C).

4.3. Sludge Quality

The high solid accumulation in the first reactor, which occurs with low hydrolysis rates, as in the case of low temperatures, can be a drawback of the two-phase system. When a one-stage UASB is employed, the excess sludge produced is rather well stabilized, whereas, when a two-phase UASB is applied, the excess sludge needs post-digestion possibly in combination with other solid wastes (e.g., vegetables, fruits, etc.) in a separate CSTR [3]. In a two-phase system, the SRT may become too low to achieve a good stabilization of the excess sludge. However, it is still possible to achieve acceptable excess sludge quality by applying the sludge stabilization in a separate heated digester [59,129]. The choice for the number of phases in a UASB system mainly depends on the required SRT and, therefore depends on the ambient temperature, temperature fluctuations and concentration, and removal and hydrolysis of suspended solids in the sewage. For treatment of total sewage under low temperature conditions, a two-phase system is surely preferable in comparison with one-stage system, whereas at high temperatures, a one-stage is more attractive [3]. For treating low-strength wastewater at medium to high ambient temperatures, two-phase UASB would be the best choice with reference to enhanced treatment efficiencies. Elmitwalli et al. [54] reported that a major portion of the excess sludge in a two-step AF→AH was produced in the first stage (i.e., AF reactor). While the settlability and dewaterability of this sludge are good, they require post stabilization. The excess sludge from the AH reactor was found to be well stabilized. At increasing loadings of suspended solids, the sludge in the AH reactor led to deterioration of the maximum specific methanogenic activity, which confirms the importance of separation of suspended solids in a first step prior to the treatment of domestic sewage in a methanogenic reactor.

5. Future Research Needs and Concluding Remarks

A two-phase UASB is expected to perform better than the one-stage UASB. Prashant et al. (2006) demonstrated, in batch experiments, the impedance caused by suspended solids or COD_P on biotransformation of organics to CH_4 [79]. Studies regarding the impact of hydrolysis, as a pretreatment of complex wastewater, in the performance of UASB for treating other COD_P, such as proteins and lipids, are lacking in the literature.

Also, COD reductions in the hydrolytic reactors were observed in the range of 11–59% (refer Table 4). COD in such systems can be lost by substrate methanogenesis. Methanogenesis in hydrolysis reactor or pretreatment unit may be due to the presence of a good amount of seed sludge (40–50%) or a low F/M (F/M < 1) and other micro-environmental parameters. The impact of hydrolysis as pretreatment of complex wastewater is expected to be not only in

the performance of UASB, but also on subsequent post-treatment and sludge disposal. Considering the literature on (i) hydrolysis, (ii) the performance of the full scale UASB process, and (iii) bench-scale two-phase treatment of sewage, a need to investigate hydrolysis of COD_P without losing carbon as CH_4 was observed. Therefore, efforts are required to limit methanogenesis in the hydrolysis unit. To achieve this, it is suggested to startup the hydrolytic reactor and to recover CH_4 from the reactor. It is also suggested that the F/M ratio in the system should be varied and pH value also monitored to reach an optimum condition where methanogens will not be encouraged in the first-phase hydrolytic reactor.

Few researchers have investigated optimum mixing strategies for increasing efficiency of hydrolysis and subsequent acidification stage of AD. Ma et al. [130] performed the characterization of dissolved organic matter and key microorganisms to understand the effect of mixing for Sewage Sludge (SS) treatment. They reported that a mixing speed of 90 rpm provides the highest efficiency for hydrolysis and acidification phase (HAP). However, the efficacy of the mixing intensity is yet to be revealed properly for the maximization between methanogenesis and HAP.

Temperature is another parameter that can play a crucial role in the acidification process in AD. Ambient temperature can have a deteriorating effect on the hydrolysis thereby significant decrease in acidification as well. Studies showed that operating AD under thermophilic condition (55 °C) can improve short chain fatty acid formation. Liu et al. [131] investigated HAP enhancement for elevated temperatures such as mesophilic (35 °C), thermophilic (55 °C) and extreme thermophilic (70 °C) conditions. They reported that the optimum temperature for acidification is the thermophilic zone, which shows 115% and 12% more efficiency than mesophilic, and extreme thermophilic zone respectively. Later, Zhang et al. [132] found similar results of increased acidification of about 15.7% due to higher temperature (55 °C) in semi-continuous reactors compared with 35 °C operating temperature.

Although the AD process has a potential to attract world-wide attention due to the production of net positive energy and other valuable byproducts such as VFA, organic fertilizer, and bioproducts [133], there is very limited research on full-scale study for the understanding of one of the key aspects of VFA production during anaerobic digestion process. However, some researchers have worked on the full-scale applications to produce VFA using chemical routes [134], but production of VFA through biological pathways from SS, municipal sludge and industrial organic wastes has yet to be investigated at commercial scale. Recently, one paper was published by Liu et al. [135] describing full-scale operation to produce VFAs. They reported the hydrolysis rate in pretreatment and the VFA yield in fermentation to be 68.7% and 261.32 mg COD/g VSS, respectively, for sewage sludge. However, for more comprehensive understanding, further research is required to explore the influence of HAP/VFAs productions and their downstream applications, such as enhancing biogas production and/or for the biological nutrient-removal process in WWTPs [135].

The application of culture-independent methods to anaerobic digesters, together with crucial complementary techniques such as imaging, isotope labeling, and chemical analyses, has provided us with understanding of microbial community composition and the function of dominant populations [136]. Metagenomics, the sequencing of bulk DNA extracted from samples, provides direct access to the metabolic potential of a microbial community [137]. Improvements in sequence throughput and bioinformatics tools have contributed to a more widespread application of metagenomics to study natural and engineered systems [136]. Application of metagenomics to understand anaerobic digestion process have enabled better perspective on the representative microbial communities, the shift in communities during the entire digestion process, and the relationship between reactor performance and microbial community shifts [137–139]. Throughout the entire single-stage anaerobic digestion of high-strength food wastewater, the abundance of phylum *Chloroflexi* decreased significantly, and the methanogenic microorganisms shifted from aceticlastic to hydrogenotrophic methanogens with high increase in the proportion of syntrophic bacterial communities [138]. Application of metagenomics has opened up a new direction to appraise the complex interconnected processes performed by microbial communities, and to understand how microbial community dynamics, interactions and functionality influence digester efficiency and stability [136–140]. Use of culture-independent metagenomics might add useful information

on how pre-hydrolysis affects the dynamics of acidogenic-acetogenic-methanogenic microorganisms communities during anaerobic digestion of sewage.

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