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Review

Approaches in Design of Laboratory-Scale UASB Reactors

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Abstract: Up-flow Anaerobic Sludge Blanket (UASB) reactors are popular tools in wastewater treatment systems due to the ability to work with high feed rates and wastes with high concentration of organic contaminants. While full-scale industrial applications of UASB reactors are developed and described in the available literature, laboratory-scale designs utilized for treatability testing are not well described. The majority of published studies do not describe the laboratory UASB construction details or do use reactors that already had developed a trophic network in microbial consortia under laboratory environment and therefore are more stable. The absence of defined guidelines for geometry design, selection of materials, construction, operation rules, and, especially, the start-up conditions, significantly hamper researchers who desire to conduct treatability testing using UASB reactors in laboratory scale. In this article, we compiled and analyzed the information available in the refereed literature concerning UASB reactors used in laboratory environment, where information on geometry and/or operational conditions were provided in detail. We utilized the information available in the literature and the experience gained in our laboratory (Sustainable Waste to Bioproducts Engineering Center) to suggest a unified operation flowchart and for design, construction, operation, and monitoring for a laboratory-scale UASB reactors.

Keywords: up-flow anaerobic sludge blanket reactors; anaerobic digestion; laboratory-scale experiment

1. Introduction

Up-flow Anaerobic Sludge Blanket (UASB) reactor is an anaerobic digester for wastewater treatment, and its operational concept can be described as a vertical up-flow pumping of liquid substrate, including wastewater or growth media, through a layer of anaerobic sludge [1–6]. Microbial consortia inside the sludge layer consume digestible components as substrate and decompose them into smaller chemical compounds [7]. Within the scope of a wastewater treatment, the goal of anaerobic digestion is a complete mineralization of organic compounds combined with the production of biogas for the purpose of energy recovery.

A distinguishing feature of UASB reactors is the formation of microbial conglomerates, where the metabolic product of one microbial group is a consumable substrate for another microbial group [8]. Such microbial conglomerates grow into spherical or bean-shaped granules over time [9–13]. The sizes of granules may vary, but typically are reported in the range 0.5 to 6 mm, where longer operation leads to larger sizes [14–16]. Granulation of sludge is promoted by the presence of microorganisms that are able to produce and secrete Exocellular Polymeric Substances (EPS) [17]. The term "EPS" includes multiple types of compounds, which serve as a glue to agglomerate microorganisms together and to add some mechanical strength to a granule [18].

A combination of developing trophic microbial connections and mechanical cementation with EPS, results in higher resilience of larger granules to sudden changes of operation conditions including a change in pH, temperature mode failure, substrate switch or inconsistency of a substrate strength and content, feeding rate fluctuations, etc. [19,20]. In some cases, granules can be disrupted due to hydrodynamic forces or inner gas pressure into several smaller fragments [12,21,22] which become cores for the formation of new granules [23].

The traditional concept of a UASB reactor, as suggested by Lettinga [10,11,24–26], is represented in Figure 1a. The substrate is pumped to a reactor through the distribution system into a bottom layer of anaerobic sludge. Equally distributed in normal cross-section of the reactor, the substrate is pushed through the sludge layer (called a "digestion zone") creating a vertical up-flow. This process is concurrent with the decomposition of organic compounds of substrate and a formation of gaseous products. Besides feeding the reactor, the continuous vertical up-flow of substrate prevents the sludge layer from clogging, keeping it afloat. However, the up-flow does wash out the unattached biomass (microorganisms, that did not start to form flocs) and small flocs/granules. The liquid part above the sludge layer (called "settling zone") serves as a vertical settler and/or coagulation column to initiate the biomass and solids retention process before the actual separation. The separation process occurs in the compartment called Gas–Liquid–Solids separator (GLSS, a.k.a. three-phase separator). GLSS is traditionally located on top of the reactor column and it starts with a baffle-shaped structure in its bottom part, which serves the purpose of collecting and re-directing the gas bubbles to the main gas collection part and preventing gas bubbles from escaping with effluent.

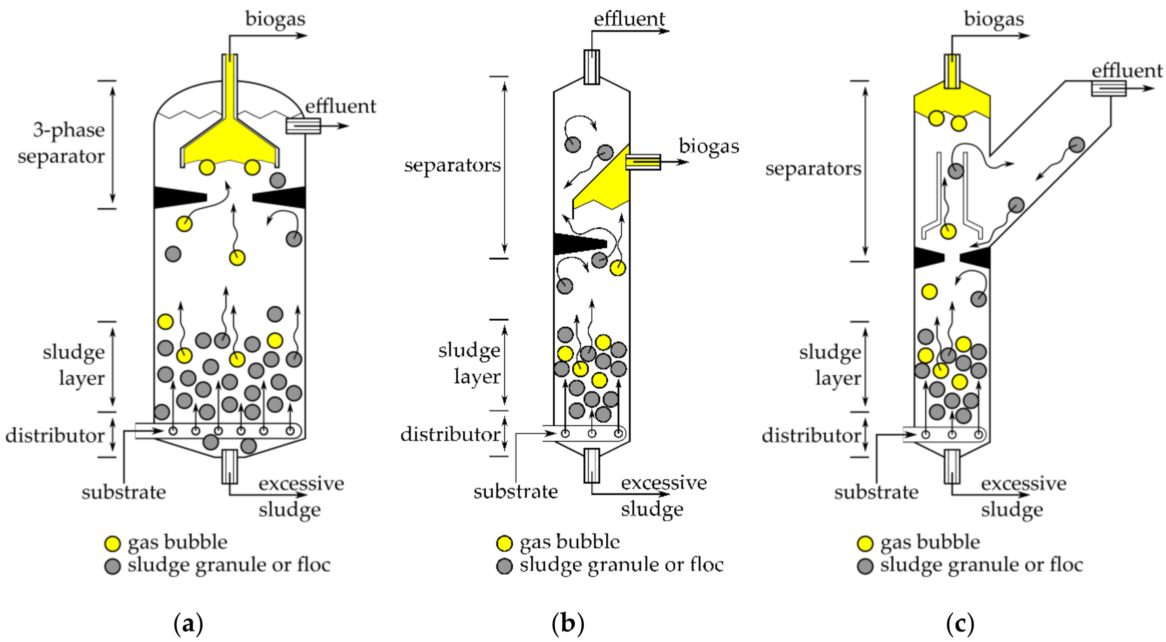

Figure 1. Operational concept of traditional Up-flow Anaerobic Sludge Blanket (UASB) reactors: (**a**) traditional; (**b**) with modified gas collector; and (**c**) Y-shaped.

The construction concept of the GLSS is shown in Figure 1a, where it's implemented via narrowing the outlet of the reaction tube with baffles. Such baffles are typically referred to as "deflectors" or "collar". The side effect of narrowing the reaction tube outlet is a creation of local velocity gradient (velocity shear), which slightly enhances the formation of granulated particles, their separation from liquid and settling back to the bottom of the reactor. Above the baffles, the GLSS contains the gas collecting structure, where the cross-section looks like a flipped upside-down funnel. In some studies, this funnel is replaced by a tubular structure with diameter larger than the distance between baffles [27,28]. The liquid is forced to flow through the space in between the lower edge of the gas collector and the baffles, to go around the funnel and leave the reactor at the effluent port.

Other existing modifications of GLSS in laboratory-scale reactors can improve the higher solids retention time, such as installing a high rate settler in headspace [29] or modification of three-phase separators [30].

In addition to the operational concept of the UASB reactor shown in Figure 1a, the same authors [11] also describe UASB reactor with a modified gas collector, which is demonstrated in Figure 1b. However, some studies [31] call such a modification of the Up-flow Anaerobic Sludge Baffled Reactor (UASBR). It may also contain the inner mechanical agitation device to prevent foam formation in the gas collecting area [32]. Recently, the Y-shaped variation of UASB reactor also became popular and is depicted in Figure 1c. In the case of the Y-shaped reactor, the GLSS is split into two individual separators: one separator is used to separate gas from the liquid and collect it directly at the top of a main tube, whereas a second collector is a sidearm tube that serves as an inclined settler for separating solids from liquid (similar to a Lamella clarifier). Use of a funnel-shaped gas-collecting element becomes optional in such case, since it serves only the purpose of preventing gas flow to an effluent side-arm.

Considering the concepts described, the optimization goal of a laboratory scale UASB reactor operation is to achieve better performance, where optimization targets for UASB performance include the following:

- Higher removal of contaminants;
- Higher biogas production rate;
- Shortening of adaptation period; and
- Resilience (robustness) of sludge.

To achieve some of those optimizations, the classical UASB concept can be combined with other types of reactors, resulting in a range of composite reactors. Some modifications are found in the literature and are presented in Table 1. This table represents options, where another reactor type is incorporated into the UASB itself, but not a sequence of two consecutive reactors.

Table 1. Existing hybrid versions of UASB reactors.

Unit to Incorporate into UASB	Resulting Reactor Name	Purpose of Incorporation	Reference
Electrolysis cell	Up-flow Anaerobic BioElectroChemical reactor (UABE)	Increase the methane production via partial capture of dissolved carbon dioxide	[33–36]
Anaerobic Filter	UASB-AF Anaerobic hybrid reactor (AHR)	Increase retention of solids inside of a reactor and prevent washout of active biomass	[37] [14,31,38,39]
Lamella settler	No Name	Increase solids retention time (SRT)	[40]

In a holistic view, the purpose of UASB reactor optimization is to keep the microorganisms in a stage of maximum substrate consumption and active growth. However, from an operational perspective, the optimization of UASB functioning is achievable via adjusting operational parameters, including, but not limited to:

- Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT);
- Recycle ratio of effluent;
- Regulation of pH;
- Retention of biomass; and
- Granulation enhancement.

Despite the long history since the invention and description of the UASB concept by Lettinga et al. [41] and increasing its application in industry, UASB laboratory scale reactors used for treatability studies are highly variable with regard to terminology, design, construction, and operation processes. This lack of uniformity leads to different results regarding water quality indicators, for example, Chemical Oxygen Demand (COD), as well as bioenergy production, for example for biomethane and biohydrogen. There is a lack of uniformity with regard to the guidelines for operation of laboratory scale conditions, which is highlighted in this manuscript and recommendation are provided for making UASB laboratory studies and results more uniform with results more transferrable among laboratories and more useful for scale up activities. These lack of uniformity with laboratory scale UASB reactors is addressed in this study and guidelines are provided for increasing the uniformity so that results are comparable across different laboratories and are also more meaningful for scale up applications of the UASB reactor process.

2. Review of Existing Solutions across Various Published Works

Despite a large number of available publications on wastewater treatment involving UASB reactors, a majority of the studies only briefly mentions constructional concepts of the reactor, and dimensional parameters are mentioned even more rarely. We collected available information on physical dimensions among existing studies in Table 2, while Table 3 shows the geometry of either hybrid reactors or where UASB reactors are installed in series with any other reactor. While building those tables we focused on the geometry of the reactor and operational conditions including substrate strength expressed as COD, Biological Oxygen Demand (BOD), etc.; loading rates; volume of reactor; and effluent recycling rates. The type of the substrate used in reported studies is provided for reference purpose only. Where Table 2 does not contain the geometric or operational parameter means that such value was not specified in the reference. Also, Tables 2 and 3 do not provide calculations based on available geometry. All information provided there is information stated in referenced publications, nothing was added. The only modifications were made to units (for COD, BOD, etc.) where they were unified across all publications.

As we can see from Tables 2 and 3, there is no uniformity in parameters of operating the UASB reactor and, and what is in our opinion even more important, information on the start-up of a laboratory UASB reactor. Such inconsistency may complicate the interpretation of results as well as the accuracy and successfulness of an experiment in general. On the larger scale, it also complicates the comparison of results obtained by different laboratories, which creates problems for feasibility studies, if the literature is the primary source of information. To be more precise, in case of a failure, incomplete information does not allow an interpretation of the data and to trace-back the reason for failure, such as unadopted inoculum or its insufficient amount, problem of biomass washout due to the geometry, problematic substrate properties, or wrong OLR or recycle ratio. Inconsistent reporting units (like OLR calculated as per total volume of reactor or per volume of digestion zone) may also harm the attempt to reproduce results of one laboratory in another one, or wrong implementation of a procedure.

Among the inconsistencies found across the studies, we also see terminology problem in the use of terms 'sludge blanket' and 'sludge bed'. The controversy of the terms is in that fact that they are:

- used interchangeably (equally)
- 'sludge bed' refers to a layer of sludge at the bottom, where it is concentrated and visually seems to be a packed layer, while 'sludge blanket' refers to a part of the reactor where sludge is swimming as flocs above the 'sludge bed'
- 'sludge bed' refers to a bottom layer of sludge, and uses 'transition zone' instead of a 'sludge blanket'

Below, in Table 4, we attempted to systematize all parameters we were able to identify in the publications reviewed. Information in Table 4 does not intend criticize, but instead the intent is to generalize and categorize information from publications referenced above.

Table 2. Overview of available information of UASB reactors used in laboratory studies.

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
1.	Hydrous ethanol vinasse COD: 121,000 $\frac{mg}{L}$ pH: 4 Ethanol: 21,007 $\frac{mg}{L}$ Acetic acid: 2237 $\frac{mg}{L}$ Propionic acid: 4304 $\frac{mg}{L}$	HRT: 6 ... 15 *days* OLR: 7.27 ... 22.16 $\frac{kgCOD}{m^3 \cdot day}$ Start-up OLR: Days 1–6: 0.34 $\frac{kgCOD}{m^3 \cdot day}$ with synthetic wastewater Days 7–8: 5.9 $\frac{kgCOD}{m^3 \cdot day}$ with substrate	*Type*: Figure 1b with added extra high rate settler above gas collecting part *Material*: Acrylic *Inoculum*: taken from already functioning UASB reactor treating vinasse of banana waster.	Cylindrical part diameter: 11 cm Cylindrical part height: 35 cm Settler basement square side: 17 *cm* Settler height: 21 *cm* Settler is installed on top of cylindrical part. Settler plates incline: 45° Operational Volume: 3 *L* No sampling ports	[29]
2.	Distillery Spentwash pH: 3.8 ... 4.2 COD: 122,000 $\frac{mg}{L}$ TS: 121,020 $\frac{mg}{L}$	HRT: 10 days OLR: 11.75 $\frac{kgCOD}{m^3 \cdot day}$	*Type*: Figure 1a *Material*: Acrylic *Inoculum*: Laboratory enriched sludge from ongoing reactor by cow dung slurry. *Seeding*: Seeding by filling the 50% of volume with sludge mixture and multiple dilution by wastewater sample.	Digestion zone: 10 × 10 × 98 cm Transition zone: 10 × 10 × 6 cm GLS zone: 19.2 × 19.2 × 25 cm Digester volume: 16.75 L Settler volume: 7.15 L GLS opening angle: 53 °C 8 sampling ports with 10 cm spacing	[42,43]
3.	Spent wash of distillery plant COD: 90,000 ... 100,000 $\frac{mg}{L}$ BOD: 30,000 ... 50,000 $\frac{mg}{L}$ pH: 3.5 ... 4.5	pH is adjusted to 6.5 ... 7.5 with lime ($Ca(OH)_2$) Dilution of substrate applied OLR: 5.63 ... 9.5 $\frac{kgCOD}{m^3 \cdot day}$ Temperature: 36 ... 40 °C Suggests to adjust the ratio COD:N:P as 300:5:1 with urea and diammonium phosphate	*Type*: Figure 1a *Material*: Acrylic *Inoculum*: active sludge from anaerobic reactor *Inoculation*: 3 L of sludge per reactor	Operational Volume: 10 L Tube I: 11.7 cm Full height: 97 cm Digestion Zone: 78 cm Several sampling ports as 5, 19, and 57 cm levels Extra Sludge washing port Deflectors angle: 55 °C GLS opening angle: 55 °C	[44]
4.	Municipal sewage	Temperature: 9 ... 32 °C HRT: 6 h OLR: 2.4 $\frac{kgCOD}{m^3 \cdot day}$	*Type*: Figure 1a Pre-existing functioning UASB reactor	Full Volume: 1148 L Height: 4 m	[45]
5.	Synthetic wastewater based on unbleached pulp mill COD: 1400 $\frac{mg}{L}$ pH: 6.3 ... 8.3	Temperature: 30 ± 1°C HRT: 30 h	*Type*: Figure 1b *Inoculum*: granulated sludge from UASB reactor treating poultry slaughterhouse effluent	Total volume: 15 L Digestion compartment: ID: 15 cm Height: 52 cm Settler cylindrical and conical compartment: ID: 15 cm Height: 30 cm	[46]

Table 2. *Cont.*

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
6.	Vanderbilt mineral medium with tetrachloroethylene COD: 3500 $\frac{mg}{L}$	Temperature: 35 ± 2 °C OLR: 10.5 $\frac{gCOD}{m^3 \cdot day}$ HRT: 0.4 day	*Type*: Figure 1a *Material*: Stainless steel *Inoculant*: Flocculent anaerobic biomass from anaerobic Continuous Stirring-Tank Reactor (CSTR) *Seeding*: 350 mL of sludge, equal to 8 g/L of TSS	Total Volume: 2 Liters ID: 9 cm Total Height: 100 cm GLS height: 15 cm Equipped with 5 sampling ports and inner heater	[47]
7.	Municipal sewage COD: 97 . . . 196 $\frac{mg}{L}$ pH: 6.8 . . . 7.2	Ambient temperature: 24 . . . 28 °C	*Type*: Figure 1a *Material*: Acrylic Sheets	Total volume: 62 L Total height: 270 cm Non-cylindrical form Sludge bed: height: 80 cm Square cross-section: 16 cm Gas collector slope: 60°	[48]
8.	Municipal landfill leachate COD: 1.5 . . . 3.2 $\frac{g}{L}$ pH: 6.5 . . . 7.0	Ambient temperature: 13–23 °C OLR: 1.2 . . . 4 $\frac{kgCOD}{m^3 \cdot day}$ HRT: 35 . . . 15 h Added $NaHCO_3$ as 0.5 g/L for neutralization purpose and no extra pH adjustment was done Recycle ratio: 3.5:1 Reports escape of methane with effluent	*Type*: Figure 1a *Material*: Stainless steel with PVC tubing and insulated with poly-urethane sheets *Inoculum*: Mesophilic anaerobic sludge from sewage treatment plant	Height: 295 cm Diameter: 13.5 GLS height: 50 cm Total Volume: 40 L Recycle ratio: 3.5:1 (feed to recycle) 2 sampling ports Contained the heater	[49]
9.	Grey water from sewer pipe COD: 647 . . . 681 $\frac{mg}{L}$	HRT: 8 . . . 20 h Ambient temperature: 14 . . . 24.5 °C	*Type*: Figure 1a *Material*: not specified *Inoculum*: sludge from anaerobic digester treating primary and secondary sludge	Full volume: 7 L Diameter: 7 cm Total height: 200 cm GLS height: 50 cm Reactor sludge filling: 100 cm	[50]
10.	Municipal wastewater COD: 672 . . . 698 $\frac{mg}{L}$	HRT: 2.4 . . . 4 h Temperature: set of reactors operating in range 12 . . . 25 °C as water bath made of PVC pipe Ø30 cm	*Type*: Figure 1c *Material*: PVC *Inoculum*: not specified	Full volume: 25 L Height: 1.35 m ID: 15 cm Inclined arm angle: 45° 4 sampling ports	[51]
11.	Sanitary waste + aerated filter effluent COD: 351 ± 166 $\frac{mg}{L}$	HRT: 6 h Experiment duration of 120 days	*Type*: Figure 1a *Material*: not specified, but either PVC or PMMA, based on provided images *Inoculum*: not specified	Cylindrical (tubular) shape Full volume: 7.8 L Total height: 60 cm Diameter: 14.8 cm 2 sampling ports GLSS opening angle: ~60° Height from top to baffles: 15 cm	[52]

Table 2. *Cont.*

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
12.	Municipal wastewater COD: 176 . . . 224 $\frac{mg}{L}$	Temperature: 20 . . . 28 °C HRT: 3 h OLR: 0.014 $\frac{mgCOD}{L \cdot day}$ or 0.009 $\frac{mgVS}{L \cdot day}$	*Type*: Figure 1a *Material*: Not specified Already existing and functioning reactors	Total Height: 3.85 m Total volume: 2.5 m^3 3 sampling ports Separate preheater of substrate before inlet point	[15]
13.	Sugar cane vinasse COD: 19,220 $\frac{mg}{L}$ sCOD: 15,300 $\frac{mg}{L}$ pH: 5.2	Temperature: 22 ± 3 °C OLR: 0.5 . . . 32.4 $\frac{kgCOD}{m^3 \cdot day}$ Up-flow velocities: 0.008 . . . 0.292 $\frac{m}{h}$ HRT: 33.33 . . . 0.86 days Recycling ratio: 1:3 Added 0.3 g $NaHCO_3$ per 1 g of COD to adjust the pH and alkalinity.	*Type*: Figure 1c *Material*: PVC *Inoculum*: Granular sludge from UASB treating poultry slaughterhouse *Seeding*: 60 L of granular sludge of VVS content 37 g/L	Total volume: 120 L Reaction zone volume: 60 L Total height: 4 m Reaction Zone Height: 2 m Diameter: 19.5 mm 8 sampling ports	[53]
14.	Mix of domestic waste with molasses (0.5:785 mix ratio) COD: 6597 $\frac{mg}{L}$ BOD: 3197 $\frac{mg}{L}$ TSS: 4500 $\frac{mg}{L}$	OLR: 6 $\frac{kgCOD}{m^3 \cdot day}$ (1.5 start-up) Temperature: 15 . . . 25 °C HRT: 10–12 h Vertical velocity: 0.5–0.7 m/h	*Type*: Figure 1a *Material*: UPVC Pre-existing reactors	Diameter: 25 cm Height: 2 m 4 sampling ports Volume: 98 L	[54]
15.	Pre-digested chicken manure pH: ~8.0 COD: 807 ± 215 $\frac{mg}{L}$ sCOD: 295 ± 46 $\frac{mg}{L}$	Feed rate; 500 mL/day/reactor + dilution with tap water HRT: 13 days Semi-continuous operation	*Type*: Figure 1a *Material*: plexiglass *Inoculum*: sludge for internal circulation reactor treating paper/cardboard industry waste *Seeding*: 1.3 L sludge per reactor (20% of working volume)	Digestion zone height: 1 m Diameter: 90 mm Volume: 6.5 L Extra sampling ports	[55]
16.	Synthetic wastewater with butyrate as a main substrate pH: 6.0–6.5 COD: 2100–15,500 $\frac{mg}{L}$	Temperature: 37 °C OLR: 4–83 $\frac{kgCOD}{m^3 \cdot day}$ HRT: 12.5–4.5 With water-jacket pH: 7.1–7.9 by addition of $NaHCO_3$	*Type*: Figure 1a *Inoculum*: flocculant sludge from anaerobic sludge digester, partially granulated in pilot-scale reactor for 2 month growing on sucrose *Seeding*: 1.5 L of adapted sludge per reactor	Digestion zone height: 50 cm Digestion zone diameter: 8.4 cm Digestion zone volume = 2.8 L Settler zone height: 25 cm Settler zone diameter: 11.4 cm Settler zone volume: 2.0 L 5 sampling ports	[27]

Table 2. *Cont.*

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
17.	Synthetic wastewater COD: 6000–20,000 $\frac{mg}{L}$ pH: 7.1–7.8 (caused by buffers in WW)	HRT at beginning: 12–1.8 h OLR: 18–260 $\frac{kgCOD}{m^3 \cdot day}$ Increasing OLR by 50% after each achieving of removal rate of 80% Preheating of substrate: 37 °C Alkalinity spiked with $NaHCO_3$ Volumetric loads calculated per digestion zone volume only	*Type*: Figure 1a *Inoculum*: anaerobic digester treating municipal wastewater *Seeding*: 6.5 L of inoculum (1.% VSS and 1.3 TSS)	Volume: 8.5 L Digestion (+ 5 L of GLS) Digestion zone ID: 104 mm Digestion zone H: 1000 mm GLS: ID 144 mm GLS: H 300 mm 7 evenly distributed sampling ports	[56]
18.	Municipal sewage pH: 4.4 COD: 531 $\frac{mg}{L}$ BOD: 359 $\frac{mg}{L}$	Temperature: 25 . . . 35 (ambient) Feed rate: 28 L/day Up-flow velocity: 0.116 m/h OLR: 1.062 $\frac{kgCOD}{m^3 \cdot day}$ pH adjusted with NaOH up to 6.7 ± 0.1 Reported granulation on 20th day for main experiment.	*Type*: Figure 1a *Material*: Glass sheets *Inoculum*: adjusted cow dung manure Inoculum adaptation: 9 L of inoculum + 1 L of nutrients, grow for 120 days growing on sucrose with $(NH_4)_2HPO_4$. Remove undigested residuals via filtering through the 3 mm mesh. *Seeding*: 4 L of filtered sludge from adaptation per reactor	Working volume: 14 L Length to height ration: 1:14 Height: 140 cm (it is not cylindrical) Length of base: 10 cm Area of reactor: 100 cm^2	[57]
19.	Distillery effluent from fermentation-based vitamin C production plant COD: 6000 . . . 38,000 $\frac{mg}{L}$ BOD_5: 2000 . . . 14,000 $\frac{mg}{L}$ pH: 4.5–6.2	35 ± 2 °C (constant temperature room) OLR: 6 . . . 11.8 $\frac{kgCOD}{m^3 \cdot day}$ Upflow velocity: 0.52 m/h HRT < 10 h 252 days of total experiment 100 days of start-up Adjusted COD:N:P as 300–600:5:1 with urea and KH_2PO_4 pH adjusted with NaOH up to 7.2	*Type*: Figure 1a *Material*: stainless steel *Inoculum*: sludge (VSS 31.0 g/L) from anaerobic digester treating the municipal wastewater	Active volume: 2.3 m^3 Height: 5.90 m Inner diameter: 0.8 m Conical shape of bottom 5 sampling ports Recycle line active	[58]
20.	High-strength distillery wastewater pH: 3.42–5.84 TS: 31,520–126,240 $\frac{mg}{L}$ TSS: 1040–26,640 $\frac{mg}{L}$ COD: 68,000–100,000 $\frac{mg}{L}$ BOD: 21,600–35,000 $\frac{mg}{L}$	HRT: 4 . . . 2d Temperature: 37 °C with water jacket OLR: 15.34 $\frac{kgCOD}{m^3 \cdot day}$ Experiment duration: 635 days Start-up: 65 days with HRT: 47.11 h	*Type*: Figure 1a *Material*: borosilicate glass *Inoculum*: the sludge from UASBR treating distillery wastewater *Seeding*: ~30% of reactor volume	Inner diameter: 92.1 mm Total Height: 79.6 cm Digestion zone: 59.97 cm Digestion volume: 5 L Water jacket ID: 132.10 mm Sampling ports: 6 GLSS opening angle: 70° (flipped funnel)	[59]

Table 2. *Cont.*

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
21.	Distillery wastewater COD: 107,000 $\frac{mg}{L}$ TOC: 39,200 $\frac{mg}{L}$	Temperature: 37 °C Flow rate: 2.2 . . . 3.3 L/d Recycle: 50% of influent flow $NaHCO_3$ added as 3 g/L to adjust pH to 7 OLR: up to 3 $\frac{kgTOC}{m^3 \cdot day}$	*Type*: Figure 1a *Seeding*: 500 mL of inoculum per reactor, + 3 L of glucose-based synthetic wastewater and nutrients, including Ca^{2+} and PO_4^{3-} to stimulate granulation	Total Height: 1.35 m Fluidization part volume: 3 L Fluidization part H: 1.05 m Fluidization part ID: 5.9 cm Settling part volume: 3 L	[28]
22.	Cane molasses vinasse COD: 10 $\frac{g}{L}$ pH: 4.1 COD: 120 $\frac{g}{L}$ BOD: 30 $\frac{g}{L}$ TS: 100 $\frac{g}{L}$ TS: 50 $\frac{g}{L}$	Temperature: 55 °C with water jacket OLR: up to 28 $\frac{kgCOD}{m^3 \cdot day}$ Experiment duration: 430 days Added 5 g/L of $NaHCO_3$ to maintain 7.3 pH all-over experiment.	*Type*: Figure 1b with extra settler above gas collector *Material*: Stainless steel *Inoculum*: sludge (12 g VS/L) from suspended growth type digester treating distillery wastewater *Seeding*: 87 L of sludge per reactor	Volume: 140 L (126 L digestion + extra for GLSS) Digestion par: 20 cm ID × 4 m height Solids separator was made of inclined plates: 60°	[60]
23.	Wastewater with high corn-starch content pH: 6.8–7.9 COD: 3000–75,000 $\frac{mg}{L}$	Temperature: 37 °C with pre-heater HRT: 24–12 *h* and OLR: 3 . . . 150 $\frac{kgCOD}{m^3 \cdot day}$ Experiment duration: 510 days OLR is calculated on the volume of digestion zone only. pH adjusted with $NaHCO_3$ equal to COD, but < 8 g/L to prevent toxicity of Na^+. Reports pH of effluent as 6.8 at the highest OLR	*Type*: Figure 1a *Inoculum*: Sludge from anaerobic digester treating sewage wastewater *Seeding*: 6.5 L of sludge per reactor	Volume: 8.5 L of digestion zone + 5.0 L GLSS Digestion ID: 104 mm Digestion Height: 1000 mm GLS ID: 144 mm GLS Height: 300 mm	[61]
24.	Recycled paper mill wastewater pH: 7.4 COD: 5330.5 $\frac{mg}{L}$ TS: 32.99 $\frac{g}{L}$ VS: 27.28 $\frac{g}{L}$	Temperature: 37 ± 2 °C with helix heat exchanger Feed: 0.5–4.5 l/h, increment by 0.5 l/d OLR: 1–10 $\frac{kgCOD}{m^3 \cdot day}$ Load calculations per digestion zone!	*Type:*Figure 1a *Inoculum*: sludge from full-scale UASB *Seeding*: 25 L of sludge per reactor	Volume: 70 L (digestion zone: 53 L) Height: 1 m (30 cm of which is GLS) Diameter: 30 cm	[62]

Table 2. *Cont.*

#	Substrate	Operating Conditions	Used Type of Reactor, Material, Inoculum, and Seeding	Constructional Geometry	Reference
25.	Distillery wastewater COD: 40.389 $\frac{g}{L}$ pH: 3.2 … 3.8	Flowrate: 18 L/d Vertical up-flow velocity: 0.0925 $\frac{m}{h}$ HRT: 15.6 h ORL: 53.75 $\frac{kgCOD}{m^3 \cdot day}$ (digestion zone) pH: 6.7 ± 0.1 with NaOH Extra mixing pump inside of reactor	*Type*: Figure 1a *Material*: PVC *Inoculum*: 18 L of cow dung and 2 L of substrate and aged for 3 weeks and filtered through 3 mm mesh.	ID: 10.16 cm (4in) Height: 142.24 cm (56 in) + 14.2 cm of GLSS Effective volume: 15.4 L D: H ratio: 1:14 5 sample ports Gas collection funnel opening angle: 55°	[63]
26.	Vinasse cane alcohol wastewater pH 4.03 … 4.44 COD: 57.59 … 128.63 $\frac{g}{L}$ TS: 17.85 … 113.98 $\frac{g}{L}$ VS: 11.81 … 58.11 $\frac{g}{L}$	Temperature: 35 ± 2 °C OLR: varied 1 … 6 $\frac{kgCOD}{m^3 \cdot day}$ HRT: 109 … 25 days Up-flow velocity: 2 … 3 $\frac{m}{h}$ Biogas cleaned with 3N NaOH solution	*Type*: Figure 1a *Material*: Glass *Inoculum*: sludge from wastewater treatment plant treating mix of urban and industrial wastewater *Seeding*: 600 mL of inoculum resulting in 10.63 g VS/L in reactor	Digestion part: 53 cm H × 7.5 cm ID Digestion part volume: 2.3 L 6 sampling ports	[64]
27.	Distiller's grains wastewater COD 16,500–22,520 $\frac{mg}{L}$ pH 3.3–4.3 VFA: 3000–3600 $\frac{mg}{L}$ VSS 190–640 $\frac{mg}{L}$	pH: ~7 with $NaHCO_3$ OLR: 3.2 … 48.3 $\frac{kgCOD}{m^3 \cdot day}$ (33.3 was optimal) No reactor heater, the substrate was preheated to 37 °C before entering the reactor Start-up OLR: Linear increase from 0.42 to 5.6 $\frac{kgCOD}{m^3 \cdot day}$ for 27 days	*Type*: Figure 1a with second level of gas collectors as on Figure 1b *Material*: acrylic Inoculum: Sludge from mesophilic anaerobic digester in sewage treatment plant *Seeding*: Seeded with 5.2 L of sludge with VSS content of 12.3 g/L, degassed by auto-incubation at 37 °C for three weeks.	6 sampling ports with spacing of 20 cm in between. Inner diameter of Tube: 8.2 cm Height: 190 cm (total), 155 cm (reaction zone) Total volume: 8.18 L Inner diameter of GLS: 14 cm OD of gas harvesting funnel: 10 cm Funnel opening angle (60°) Duration of experiment: 420 days	[65]

Table 3. Information on UASB modifications of multi-step reactors involving UASB.

#	Substrate	Operating Conditions	Type, Material, Inoculum, Seeding	Geometry	Reference
1.	Distillery spent wash pH: 4 ... 4.5 COD: 80,000 ... 12,000 $\frac{mg}{L}$ TS: 60,000 ... 85,000 $\frac{mg}{L}$ BOD_5: 35,000 ... 45,000 $\frac{mg}{L}$	Temperature: 20 ... 40 °C (ambient) pH: ~7 with $NaHCO_3$ Substrate COD:N:P as 100:5:1 with NaH_2PO_4 and Urea OLR: 1.0 ... 8.0 $\frac{kgCOD}{m^3 \cdot day}$ (start-up), 36 $\frac{kgCOD}{m^3 \cdot day}$ HRT: 6 ... 48 h Observed granulation at day 50.	Type: Figure 1a with packing materials. So called Hybrid UASB reactor Material: PMMA Inoculum: flocculent sludge from anaerobic digester of sewage treatment plant Seeding: sieved through 1 mm mesh, loaded as 15 g VSS/L (2.5 L per reactor)	Operational liquid volume—5 L (45 cm of total height) Diameter: 10 cm Overall height: 77 cm GLS separator was replaced with packing, taking 19 cm of total height (volume 1.5 L)	[14]
2.	Tannery wastewater COD: 8600 ... 14,100 $\frac{mg}{L}$ pH: 2.8 ... 3.7	Temperature: 17 ... 38 °C (ambient) Substrate was diluted to COD value of 5400 ... 9400 $\frac{mg}{L}$ Experiment duration of 52 weeks Equalization tank (600 L) prior to 1st stage UASB Start-p OLR: 24 h HRT: 5 ... 24 h	Type: two reactors as Figure 1a in line Material: UPVC	Volume: 94 L Total height: 325 cm Digestion zone height: 240 cm Tube ID: 20 cm 5 sampling ports every 55 cm Funnel overlap on baffles: 1.5 cm per side	[66]
3.	Molasses-based ethanol distillery wastewater	HRT: 70 h, treating as 2nd stage after CSTR Feed flow: 3.4 L/d Temperature: ambient	Type: Figure 1a	Digestion Volume: 10 L Digestion ID: 0.08 m Digestion Height: 1.5 m	[67]

Table 4. Summary of the geometry and operational parameters for existing UASB reactor designs.

Criteria	Options/Area of Application/Observations
Height	No constraints on height. The smallest found reactor was 30 cm tall, the largest as above 4 m. Perhaps, limited only by the available space in a laboratory.
Volume	Small volumes are 0.5, 0.75, 1, and 2–2.4 L. Larger volumes of 14 and 55 L were also found. Usually, reactors with volume greater than 1 m^3 are referred as pilot-scale.
Height: Diameter (H:D) ratio	Since the substrate has an up-flow velocity, the reaction part of UASB reactor in some degree functions as a sedimentation or coagulation column, where ratio H:D should help preventing the biomass washout [19]. This parameter is very rarely reported, and reporting of it can be confusing due to not clear geometry reference. There are reports of H:D ratio as a diameter of a reactor to either a total height of a reactor or to the height of a reactor without GLS. We see reasonable to calculate it as a diameter of reactor to the height without GLSS, since the goal of GLSS is to create a chamber for gas capture above the reaction tube of a reactor. From review studies, such ratio for majority of cases is in range from 8 to 14. However, there are also extreme cases as 3.5 or 23.
Construction material	For small volumes (up to couple liters): Borosilicate glass For small and medium sizes: PVC and PMMA For pilot scale: Stainless steel.
Gas–Solid–Liquid Separator (GLSS, Three-Phase Separator)	The particular design varies with the concept of the reactor itself, and options can be split into: Implementation of baffles Gas collection For tubular reactor designs, the deflectors are typically made as an O-rings with a triangular cross-section. For rectangular reactors, a series of inclined baffles are installed to narrow a main liquid flow. For smaller reactors, baffles are sometimes omitted, probably, because it's difficult to implement those in smaller volumes. Another case when deflectors were noticed to be omitted is when GLSS is represented by a separate part (either tube or funnel), wider than the major reaction tube, and a diameter of a gas collector is close to a diameter of a reaction tube. Gas collector is usually represented by a flipped upside-down funnel for smaller reactors. For larger ones, it can be a separate compartment. Y-shaped reactors do not have any specific structure inside.
Heating	Among the reviewed designs the following heating systems were noticed: Heating pads or tapes Water jacket No heating Inner heaters (helix shaped) Water jacket is the most common option for smaller designs but it complicates the placement/insertion of sensors (like pH, ORP, temperature, etc.) into a reactor. Larger reactors usually use heating pads or a combination of heating pads with thermal insulation material.
Temperature ranges	Mesophilic: 35 ± 2 °C or 37 ± 1 °C Thermophilic: 55 ± 1 °C Ambient temperature Ambient temperature with thermostat to prevent overcooling
Inoculum material	No constraints: Granular or flocculated sludge from another UASB Non-granulated anaerobic or active sludge Adjusted inoculum from non-sludge sources, like animal manure
Seeding (inoculating)	Across the reviewed studies, this was the most inconsistent parameter, which was not even always reported. The process was reported as: (a) filling reactor with raw sludge up to a certain percentage of height; (b) volumetric load of sludge per reactor, sometimes mentioning its VSS and/or TSS equivalent; and (c) final concentration of sludge in reactor as TSS or VSS. Also, few studies suggested to sieve the sludge through 1–3 mm mesh to remove any undigested particulate or residuals before seeding.

Table 4. *Cont.*

Criteria	Options/Area of Application/Observations
Substrate preparation, feeding and pH management	Few studies considered the adjustment of substrate based on ratio COD:N:P. However, the final ratio does not match across publications and varies for COD parameter 300–600:5:1. Surprisingly, no-one mentioned adjusting the C:N ratio, which is recommended for anaerobic treatment in general. Only one publication mentioned the addition of compounds to stimulate granulation.
Substrate pH management	Researchers use either pH adjustment in substrate directly or pumping pH adjusting solution to the reactor. Used adjusting compounds are either hydroxides or bicarbonates. Interesting fact: addition of 0.5–3 g of $NaHCO_3$ per 1 L of substrate was sufficient to maintain a stable effluent pH around 7. In some extreme cases 8 g per 1 L of substrate were sufficient to work with OLR 150 $\frac{kgCOD}{m^3 \cdot day}$.
OLR and HRT	HRT and OLR are interdependent values and both are optimization points in research. Researchers aim to increase OLR and decrease HRT. These parameters are points of inconsistent reporting: Some sources report OLR and HRT as referred to the total volume of the reactor (both reaction tube and GLSS) Some sources report OLR and HRT as referred to the volume of the reactor without the volume of GLSS Higher limit for OLR is not specified, since it depends on chemical composition of influent wastewater and its strength.
Substrate distribution system	Typically is not reported, but where it is mentioned it's either: a circular tube with evenly distributed outlet holes and an inlet from the side through the wall of reactor an inlet into conical-shaped bottom of reactor a side inlet through the wall into bottom compartment with inclined bottom

3. Discussion

Studies, involving the UASB trials, are usually purposed for: (a) treatability testing and energy recovery estimation; (b) microbiology studies on changes in microbial consortia during adaptation to new substrates or long-term operation for further modeling of trophic network; or (c) toxicity and granulation process studies. In the scope of this manuscript, we would like to identify the common needs of such research and point out the differences, where it is important. Here, we would like to focus on experimental aspects, which are needed to pay attention to, while designing the reactors and its infrastructure.

3.1. Volume of Reactor

The first thing that affects the final volume of a designed reactor is the available amount of sample/substrate. Some samples of substrates are available in very limited quantities due to the policies of supplier companies or may be a subject of special regulations preventing the dumping of effluent to a sewer (Ex. industrial wastewater). Depending on the complexity of substrate and potential inhibitory effects, the reactor start-up period might occupy a substantial period up to 120 days [14,57,59,65,68], thus the volume of a reactor should allow to utilize the available sample volume for both start-up period and experiment duration.

3.2. Material of the Reactor

Due to the specifics of laboratory studies, the reactor needs to be constructed with the feature to visually inspect the content. It allows one to: (a) confirm the fact of granulation and (b) inspect the foam or scum formation, etc. This significantly narrows the selection choice of materials, limiting it to (a) polymethyl methacrylate (a.k.a., PMMA, Plexiglas, Perspex, acrylic glass), (b) borosilicate glass, and (c) clear polyvinyl chloride. Each of the mentioned materials can be used, and in our opinion it is more of a question of budget and available stock parts. We compare pros and cons of each material in Table 5.

Table 5. Comparison of materials used for UASB reactors in various studies.

Material	Pros	Cons
PMMA	Less expensive than glass Almost no film formation (unless scratched) Optically clear, may have some UV-protective coating Stronger that glass Machinable with mechanical tools (CNC/lathe/mill/drill) Cracks can be fixed with either solvent treatment, epoxy of UV-curable resin in short time frame	Needs machining equipment If sterilization is needed: consider chemical sterilization Easily scratchable
Glass	Optically clear Non-UV degradable, chemically inert (under conditions of AD) Washable Autoclavable	Expensive In case of cracks becomes sensitive to vibrations and not usable Requires specialist (glass blower) to build or repair/fix Fragile
PVC	Clear, but not optically. Has blueish color Machinable, but melts easily Relatively cheap and available on the market, has a wide set of existing fittings for quick assembly	Degrades over time, becomes fragile Non-UV-stable, becomes yellowish over long-term expose to light containing UV spectrum (sunlight) Microorganisms form biofilm on its surface

Borosilicate glass is an excellent option if used for studies with sterile cultures, since it can be autoclaved. However, in the author's opinion, the ideal reactor must be manufactured of stainless steel and be featured with an inspection window, a water jacket and multiple sampling ports. Such a design would be chemically resistant under conditions of anaerobic digestion, autoclavable, and meet multiple research needs. However, such construction complicates the customization and should be done for optimized and fully tested design after confirmation of its efficiency. The authors currently use PMMA due to machinability of this material, its transparency, and stability under conditions of anaerobic digestion (AD).

3.3. Heating of Reactor

Heating of reactor under laboratory conditions is defined by: (a) actual need for heating and (b) necessity of sampling the content of reactor and location of sampling. If no sampling of reactor content is needed, the water jacket would be the most suitable option. Otherwise, sampling ports complicate the construction of water jacket. Without a water jacket, consider: (a) use of heating tapes or flexible heating pads or (b) preheating of substrate and thermal insulation of reactor to keep the temperature.

Heating tape on the outer surface of PMMA or PVC reactor is not recommended, since it could cause local damage, when the contact point of wall material and heating tape is locally overheated, causing melting or other types of damage. Our laboratory experienced problems with heating tapes even under mesophilic conditions. The reactor that got damaged, was controlled by thermostat with an external submergible temperature sensor. The damage consisted of the tape melting through the wall of the reactor causing leakage. Thus, we moved to a water jacket in our projects.

Perhaps, the use of heating pads would be more secure due to a larger area of contact and, hence, more uniform heating. Extra uniformity may be added by use of heat-transfer pastes, but they will decrease the observability of the process in reactors. However, it is still a viable option when there is a need for the presence of sampling ports on various levels or there is no way to implement a water jacket due to other reasons.

3.4. Inoculum: Preparation, Adaptation, and Seeding

While the granulated anaerobic sludge is the desirable inoculum, authors fully realize the probability of a situation when researchers do not have a source for granulated sludge. In such a case, the manure sample of animal origin could be a source of methanogenic microbial consortia, and referenced studies [27,42,43,57,63,65] suggest self-digestion of such sample or mixing it with a substrate and conditioning for up to 3 weeks. The presence of methanogenic microorganisms is required for the generation of methane, but not every manure contains methanogens. The most typical confirmed cases of manure containing methanogens are cattle and swine manures. The presence of methanogenic bacteria could be confirmed by conducting specific methanogenic activity test [69,70], which is very close in technique to a popular Bio-Methane potential (BMP) test [71], but conducted on a nutrient media containing acetate as the only source of carbon [72].

Some studies suggest the sieving of inoculum through a 1–3 mm mesh to remove undigested or large inert material. It is reasonable, if the inoculum originated from manure, since manure samples may contain some animal bedding, or sewage wastewater treatment facility, which may contain hair, etc. However, if the sample originated from an industrial wastewater treating facility, such sieving could be optional, especially if sludge is already granulated and granules are large. Also, the effect of exposing sludge or granules to air during the sieving is not clear. Perhaps, the sieving process should be done in anaerobic chamber.

The seeding of reactor must be calculated and expressed as Volatile Suspended Solids (VSS), introduced with the inoculum, per working volume of reactor according to [73,74] and seeding should be in the range 10 to 20 $\frac{kg_{VSS}}{m^3}$, (however, it also could be up to 25$\frac{kg_{VSS}}{m^3}$) [10]. Inoculum should be analyzed for Total Solids, (TS), Volatile Solids (VS), Total Suspended Solids (TSS), and Volatile Suspended Solids (VSS) since sludge is also characterized by VS:TS and VSS:TSS ratio, as criteria of alive biomass if condition of sludge is tracked over time [75] and ratio VSS:TSS of sludge in range of 0.7 to 0.85 is likely to cause granulation [59]. The recommended method for solids content analysis is specified in Standard Methods 2540 [76].

3.5. Substrate Adjustment

Before any adjustments is done to a substrate, the treatability can be roughly characterized by the ratio BOD_5:COD, which is referred to as a biodegradability index (BI) [77,78]. For municipal raw wastewater BI is usually in the range 0.4 to 0.8 [79,80] and it is considered to indicate good treatability. Greater index means better bio-treatability and pretreatment can increase the value of BI [81,82]. Estimation of sample degradability based on BOD_5:COD is inconsistent, but can be generalized as: (a) highly bio-treatable if greater than 0.5; (b) bio-treatable if greater than 0.3; and (c) not bio-treatable when lower than 0.2 [81–85].

One of the primary adjustments for substrate is the C:N ratio [86] by mass, with the optimum in the range 25 to 30 [87–89] or 20 to 30 [90] and higher temperature ranges require higher C:N ratio. However, it also could be a substrate-specific optimization parameter [91–93]. Some authors also consider C:P ratio for methanogens between 16:1 and 75:1 [94,95] as optimal, while C:N:P ratio is considered to be favorable in the range 400:5:1 to 100:28:6 [95,96]. Some inconsistence to in attempt to meet those ranges may come from measurement techniques, where various authors use either: (a) elemental analyzers [97] or (b) total carbon and Total Kjeldahl Nitrogen (TKN) [90]. Across referenced in this manuscript studies, following compounds were used to correct ratio: KH_2PO_4, NaH_2PO_4, $CO(NH_2)_2$ (urea or carbamide), and $NH_4H_2PO_4$.

Individual studies stated the need to account for sulphur [98], and report C:N:P:S ratio as 600:15:5:3 to be the optimal for methanization [99]. Perhaps, such increase of considered elements is reasonable, since elemental composition of anaerobic biomass is reported as $C_5H_7O_2NP_{0.06}S_{0.1}$ according to [100–102], but not yet widely used in anaerobic digestion studies.

The ratio of COD:N:P of 250:5:1 is generally suggested for anaerobic treatment [103–105], however, some variation exist between 900:5:1.7 and 150:5:1 [104,106,107] and could be even 300:1:0.1 [108]. Other studies recommend 300:5:1 as start-up conditions specifically for UASB [75,96,109,110]. Important to mention, that "N" in such proportion refers to the total nitrogen [108]

pH adjustment for methanogenic bacteria should bring the pH in the optimal range 6.8 to 7.5, while outside of the 5 to 8.5 range, methanogenesis is fully suppressed [111–113]. However, for anaerobic digester the range of 6.8 to 7.2 is recommended, due to widely used in wastewater treatment lime as pH adjusting chemical [114]. Across referenced studies we noticed NaOH and $NaHCO_3$ as widely usable compounds to adjust pH, however, the choice is wider [115].

3.6. Granulation Stimulation

If granulation enhancement is needed, the Ca^{2+} in concentration 100–200 mg/L of substrate can be added [116], or even 150 . . . 300 mg/L at the start-up [117,118]. The role of calcium in granulation process is not clear, but it is assumed to form precipitates with carbonate and phosphate [21,119]. Use of Mg^{2+} is not recommended, since it causes disaggregation of granules [120], even though it is expected to precipitate as $MgNH_4PO_4$ [10]. Normally, granulation should be observed within 4–6 weeks after the start of the experiment [73].

3.7. Start-up Feeding

The original research of [73] recommends the OLR as 0.05 . . . 0.10 $\frac{kg_{COD}}{kg_{sludge\ VSS} \times day}$ for the start-up period and increasing of OLR after achieving the removal rate of at least 80%, however, the increment values are not specified. The expression of COD load per VSS of sludge per day is called "sludge load", but in studies OLR is usually reported as $\frac{kg_{COD}}{m^3 \times day}$, which is called "space load". Based on the previously suggested inoculum seeding as 10 . . . 20 $\frac{kg_{VSS}}{m^3}$, the start-up space load should be in range 0.5 . . . 2 $\frac{kg_{COD}}{m^3 \times day}$, however exact calculation based on the loaded VSS of inoculum must be done. The vertical velocity of the substrate is suggested not to exceed 0.5 . . . 1 $\frac{m}{h}$ [10] in general, but minimal values are not reported and no details were found for the start-up period.

3.8. Infrastructure of UASB Reactor

Based on our experience and referenced here studies, we want to suggest a unified operational process flow diagram as in Figure 2, where we would like to emphasize several aspects.

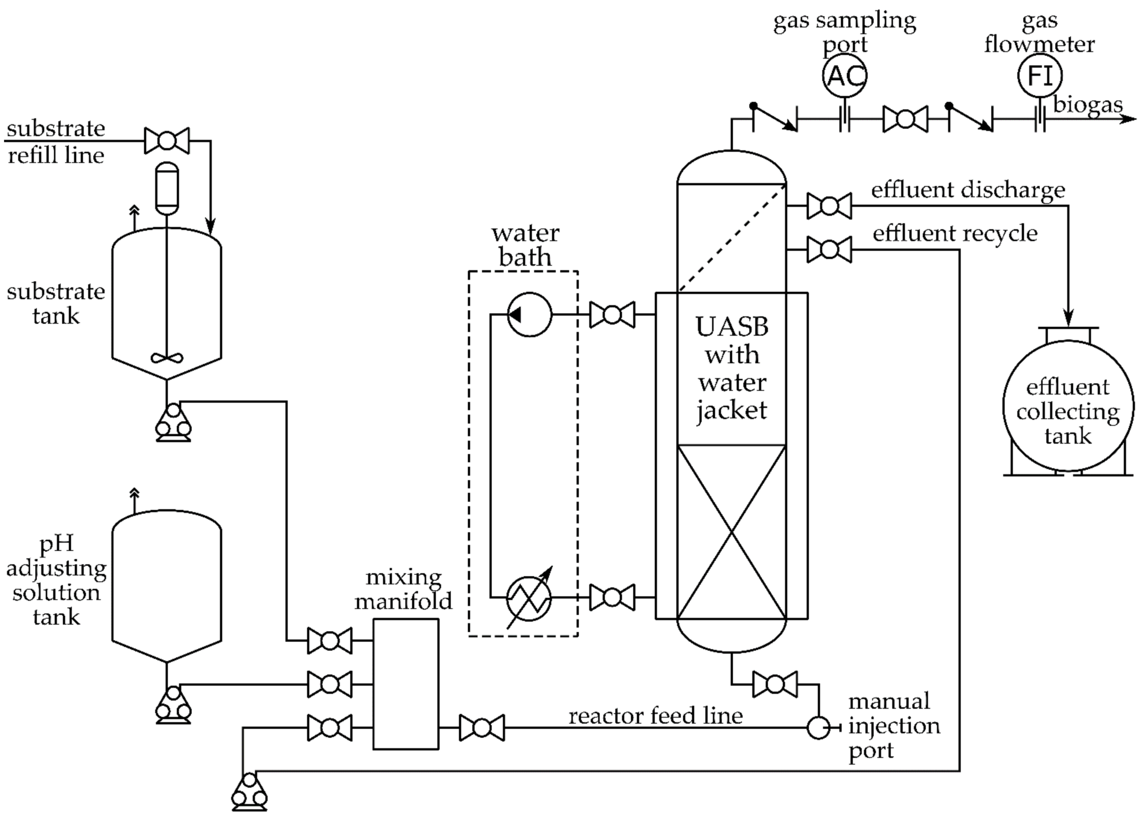

Figure 2. Recommended flowchart of UASB infrastructure set-up.

3.8.1. pH Adjusting and Alkalinity

As mentioned above, the composition of pH adjusting solution is a point of choice [115], but, regardless, the solution should be pumped directly into the reactor feeding line (the mixing manifold on schematic of Figure 2.

Otherwise there is a potential for growth of competitive microorganisms in the substrate feeding tank, leading to chemical changes of substrate. The concentration of pH adjusting solution should be balanced based on: (1) daily amount of solution needed to pump (pumps may have lower limit of pumping speed) and (2) minimizing substrate dilution. The interim decision could be to use concentrated solution that is pumped and dosed on a timer, if calculated flowrates are below the limits of a pump. High concentrations of pH adjusting solutions can be chemically aggressive. To prevent the contact between the substrate and parts of pumping mechanism, the peristaltic pumps are recommended for use.

Referenced here studies dissolve or add 0.5–3 g of $NaHCO_3$ per 1 L of substrate and achieve the stable pH in favorable methanogenic range. However, there are more general suggestions to maintain the ratio between alkalinity of substrate (expressed as $CaCO_3$) and its COD as 1.2–1.6 $\frac{g\ CaCO_3}{g\ COD}$ [121]. This value can be used as a reference to calculate the dosage portions and intensity for pH adjusting solution, but should be optimized later [122] downwards. Methanogenesis could occur until ratio of 0.8 $\frac{g\ CaCO_3}{g\ COD}$ with some extreme cases of 0.3 $\frac{g\ CaCO_3}{g\ COD}$, but lower values should inhibit methanogenesis and stimulate the formation of hydrogen [123,124].

Another reference value for regulation of alkalinity is ratio of Volatile Fatty Acids (VFA) to Total Alkalinity (TA). Industrial guidelines [114] recommends the ratio VFA:TA to be below 0.35 and consider the value below 0.25 as best for anaerobic digesters, and below 0.15 as safe against pH changes in substrate. The VFA should be expressed as equivalent concentration of acetic acid in $\frac{mg}{L}$ and TA as equivalent of $CaCO_3$ in $\frac{mg}{L}$. The determination of alkalinity by titration is described in Standard methods 2320 [76], as well as appropriate methods for VFA with gas chromatography is covered by Standard methods 5560. However, there were attempts to substitute VFA determination by titration [125–127], to avoid using of gas chromatograph.

3.8.2. Feeding and Recycling

The feeding of reactor, based on OLR and HRT is the point of optimization targeting the maximum achievable loads, however, the [10] recommends to limit the vertical velocity of liquid depending on the type of sludge and type of waste:

- Granular sludge + soluble wastewater: 3 $\frac{m}{h}$ continuous, up to 6 $\frac{m}{h}$ peak for a couple of hours per day;
- Granular sludge + partially insoluble water: 1–1.25 $\frac{m}{h}$, up to 2 $\frac{m}{h}$ peak for a couple of hours per day; and
- Flocculent sludge: 0.5 $\frac{m}{h}$ continuous, up to 2 $\frac{m}{h}$ peak.

After sludge matured and granulated, the flow could be increased by 50%. In the case of insufficient vertical velocity and to prevent clogging, the recycle line can be used to manage it and (a) to dilute substrate with treated wastewater, (b) to reuse of alkalinity [28], or (c) enhance the granulation by increasing the vertical up-flow [128].

Important remark: effluent recycle port must be separate and located below the effluent discharge port. It is made to prevent back-pumping of air from the effluent discharge line. In our laboratory set-up, we used the flow splitters on effluent port to obtain a recycle line, and we noticed some gas bubbles in it.

3.8.3. Manual Injection Port

A manual injection is strongly recommended and is purposed for:

- urgent (emergency) injection of solution for managing pH, coagulation/flocculation, or granulation agent problems;
- testing an enhancement of inoculant via injection of specific microbial culture(s); and
- sampling of substrate which is supplied to a reactor after all mixing procedures.

3.8.4. Biogas Collection and Counting

Notice the installed one-way valves in the gas line in Figure 2.

Check valves are important to prevent the back flow of gas and there are several reasons for that particular phenomenon:

- drop of the ambient temperature and, consequently, gas compression in gas lines according to the combined gas law;
- at the beginning of UASB operation, when substrate gradually fills the reactor and gas tubes have residual air. The oxygen from residual air is consumed and thus the volume shrinks.

Any of those reasons can lead to one of the two undesirable consequences:

- ingress of liquid from reactor to a gas line, which potentially grabs the foam and clogs the pipeline.
- if water displacement gas counter is used: backflow of liquid from counter back to a reactor.

Important clarification is to use check valves with low cracking pressure. 'Cracking pressure' is a pressure value when check valve starts opening (passing gas through itself) and this pressure (converted into inches of water column) must be taken into consideration when designing the gas separator, specifically, the height and the level difference between gas collecting part and the effluent release port. Usage of valve with high cracking pressure result in need to build tall GLSS, increasing the material needed to build reactor and its dead volume.

We also want to stress that the gas counter working on the water displacement principle is the only option for raw biogas. There are gas counters working on heat transfer principle (similar to thermal conductivity detectors of gas chromatographs), which seem to be cheaper options, but they should not be used. Those counters can be calibrated for gas flow with constant content only, which is not a case for biogas. However, they can be theoretically applied if biogas was stripped with alkaline solution to remove acidic gasses (carbon dioxide, hydrogen sulfide, etc.) and assumed to be upgraded to bio-methane. We do not recommend the use of that.

3.9. Tracking Operational Parameters

The exact set of trackable parameters depend on the purpose of a particular study, but for general cases, we listed those parameters in Table 6.

Table 6. Minimal list of parameters for tracking during UASB experiments.

Parameter	Measurement For	Used For
COD	Influent	Calculate the degradability rate of substrate
	Effluent	Calculate corrected OLR
		Reference for energy yield calculation and substrate utilization rate
pH	Influent	Estimation how favorable are conditions for methanogenesis
	Effluent	Tracking the changes of substrate
	Reactor entrance	: The actual pH value in a sludge layer if recycle line is used
Gas	Yield volume	Estimation of yield per unit of substrate
	Content	Calculation of energy recovery
		Balancing COD on biomass growth
Flowrate	Feed rate	Calculation of OLR, HRT, up-flow velocity
	Recycle rate	

These parameters are already enough to calculate the main operational parameters specified in Table 7 [10,91,129,130]:

Table 7. Main operational parameters of UASB.

Parameter	Equation	
Substrate utilization rate	$U = \frac{COD_{influent} - COD_{effluent}}{HRT \times VSS_{sludge\ in\ reactor}}$	(1)
Removal efficiency	$E = \frac{COD_{influent} - COD_{effluent}}{COD_{influent}} \times 100\%$	(2)
Hydraulic retention time (HRT)	$\theta = \frac{working\ volume\ of\ reactor}{volumetric\ flowrate\ of\ influent}$	(3)
Organic Loading rate (space load)	$OLR_{space} = \frac{volumetric\ flowrate\ of\ influent \times COD_{influent}}{working\ volume\ of\ reactor}$	(4)
Organic loading rate (sludge load)	$OLR_{sludge} = \frac{volumetric\ flowrate\ of\ influent \times COD_{influent}}{volatile\ suspended\ solids\ of\ sludge\ in\ reactor}$	(5)
Up-flow velocity	$v = \frac{influent\ flowrate + recycle\ flowrate + adjusting\ flowrate}{area\ of\ horizontal\ crosssection\ of\ reactor}$	(6)

In addition, the track of biogas composition during the UASB experiments, the total gas yield and methane yield should be logged. Mentioned above parameters for logging and calculations on their basis do provide a basic understanding of ongoing process inside of UASB reactors, while interpretation of calculations result are not the scope of this manuscript to avoid swelling of it. However authors feel also a need to mention, that if some deeper understanding of chemical process or COD balancing is needed, other researchers [59,131–133] suggest calculation of what part of metabolism is presented by certain process according to the equations, collected in Table 8:

Table 8. Equations for metabolic ratios estimation.

Parameter	Equation	
Hydrolysis	$H = \frac{COD_{CH_4} + sCOD_{effluent} - sCOD_{influent}}{COD_{influent} - sCOD_{influent}} \times 100\%$	(7)
Acidification	$A = \frac{COD_{CH_4} + COD_{VFA-effluent} - COD_{VFA-influent}}{COD_{influent} - COD_{VFA-influent}} \times 100\%$	(8)
Methanogenesis	$M = \frac{COD_{CH_4}}{COD_{influent}} \times 100\%$	(9)
COD mass balance	$COD_{influent} = COD_{accumulated} + COD_{biogas} + COD_{effluent}$	(10)

Other parameters, not included here, belong to some partial cases of UASB experiments and are subjects of individual consideration. Examples for a category of such specialized studies could be effects of salinity or metal ions on the process inside of UASB, which would require extra electrical conductivity, ion-selective electrodes, or other quantitative measurements for both influent and effluent [134,135]. If the study is dedicated to toxicity or biodegradation of particular compound, the appropriate assay tests for that compound or its metabolites should be added [136,137], etc.

4. Conclusions

With this article we would like to draw the researcher's focus towards the need to report in their publications more information on materials and methods, including specifically sketches/operational flowcharts, seeding conditions, inoculum sources and pre-treatments, and all adjustments to the substrate and feeding equipment. The consideration and addition of these details will help to facilitate a strong scientific and engineering community with comparable research results and conditions. Such detailed data and methods reporting will also significantly propel modeling studies that aim to realistically predict bioreactor behavior in various process conditions.

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Abbreviations

AD	Anaerobic Digestion	sCOD	Soluble COD
BOD	Biological Oxygen Demand	SRT	Solids Retention Time
COD	Chemical Oxygen Demand	TA	Total Alkalinity
CSTR	Continuous Stirred-Tank Reactor	TKN	Total Kjeldahl Nitrogen
EPS	Exocellular Polymeric Substances	TOC	Total Organic Carbon
GLSS	Gas–Liquid–Solids Separator	TS	Total Solids
HRT	Hydraulic Retention Time	TSS	Total Suspended Solids
ID	Inner diameter	UASB	Up-flow Anaerobic Sludge Blanket
OD	Outer diameter	UPVC	Unplasticized Polyvinyl Chloride
OLR	Organic Loading Rate	VFA	Volatile Fatty Acids
PMMA	Polymethyl methacrylate	VS	Volatile Solids
PVC	PolyVinyl Chloride	VSS	Volatile Suspended Solids

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