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Review

Critical Assessment of Hydrogen and Methane Production from 1G and 2G Sugarcane Processing Wastes Using One-Stage and Two-Stage Anaerobic Digestion

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Abstract: Sugarcane is a lignocellulosic crop which is used to produce sugar in sugarcane processing industries. Globally, sugarcane processing industries generate solid and liquid wastes amounting to more than 279 million tons per annum and by-products; namely, trash, bagasse, mill mud, and molasses. The valorisation of waste and by-products has recently increased and is playing a significant role in achieving policies and goals associated with circular bioeconomy and sustainable development. For the valorisation of sugarcane processing industry waste and by-products, a number of technologies are well established and in use, while other innovative technologies are still ongoing through research and development with promising futures. These by-products obtained from sugarcane processing industries can be converted into biofuels like hydrogen and methane via anaerobic digestion. Molasses belongs to the first-generation (1G) waste, while trash, bagasse, and mill mud belong to second-generation (2G) waste. Various studies have been carried out in converting both first- and second-generation sugarcane processing industry wastes into renewable energy, exploiting anaerobic digestion (AD) and dark fermentation (DF). This review emphasises the various factors affecting the AD and DF of 1G and 2G sugarcane processing industry wastes. It also critically addresses the feasibility and challenges of operating a two-stage anaerobic digestion process for hydrogen and methane production from these wastes.

Keywords: trash; bagasse; mill mud; molasses; anaerobic digestion; dark fermentation

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1. Introduction

The major biomass generated in sugarcane processing industry are trash, sugarcane bagasse, mill mud, and molasses. Around 279 million metric tonnes of sugarcane waste are produced worldwide each year by the sugarcane processing industry [1]. Uncontrolled sugarcane waste disposal can have serious negative effects on the environment and, consequently, human health [2]. Figure 1 illustrates the schematic representation of a sugarcane processing industry.

These wastes are solid or semi-solid in nature and can be divided into two groups: Waste from the sugar mill is characterized by bagasse, press mud/mill mud (remaining cake from juice filtration and sludge from juice settling) and molasses. Leaves and cane tips symbolise waste from the harvesting process (trash) [3]. Molasses belongs to the first-generation (1G) waste, while trash, bagasse, and mill mud belong to second-generation waste (2G) [3,4]. Many conventional sugarcane processing industries have recently been transformed into bio-refineries or second-generation sugarcane processing industries, where an assortment of waste products can be used to produce valuable products for additional revenue generation [5]. The sugarcane processing industries use co-generation technology to utilize the bagasse to produce heat and electricity to run various unit operations [6]. The cane trash generated is sometimes used as a garden mulch or soil conditioner. The mill mud is used as

a fertiliser in cane fields [7–9]. Although there are promising future developments, certain techniques for recovering waste from the sugarcane processing industries are already being used extensively, while others are still in the research and development phase. These organically rich substrates are converted into renewable energy using a variety of methods, including fermentation, gasification, pyrolysis, and anaerobic digestion [10–13]. Among them, anaerobic digestion (AD) and dark fermentation (DF) are deemed as an environmentally sustainable and well-established bio-chemical route to utilise these organic wastes for renewable energy generation [14]. Hydrogen (H_2) and methane (CH_4) can be created from sugarcane processing industry wastes via DF and AD. Various studies have been carried out on converting both first- and second-generation sugarcane processing industry wastes into renewable energy exploiting anaerobic digestion and dark fermentation. From Figure 2, it is evident that both 1G and 2G wastes were exploited in methane and hydrogen production via AD and DF processes, and a total of 52 articles were published in this sector from 2012 to 2022. However, bagasse was the most used substrate for this purpose. Moreover, no concrete article was observed which summarises all these studies and discusses the parameters affecting both the 1G and 2G wastes for methane and hydrogen production. Thus, this work aims to analyse the factors that affect hydrogen and methane production from sugarcane processing industry wastes via DF and AD. Moreover, it critically summarizes the studies that have been carried out in this sector and compares the hydrogen and methane yield form 1G and 2G sugarcane processing wastes. Acute assessments of single-stage vs. two-stage AD of 1G and 2G sugarcane processing industry waste products have been also carried out.

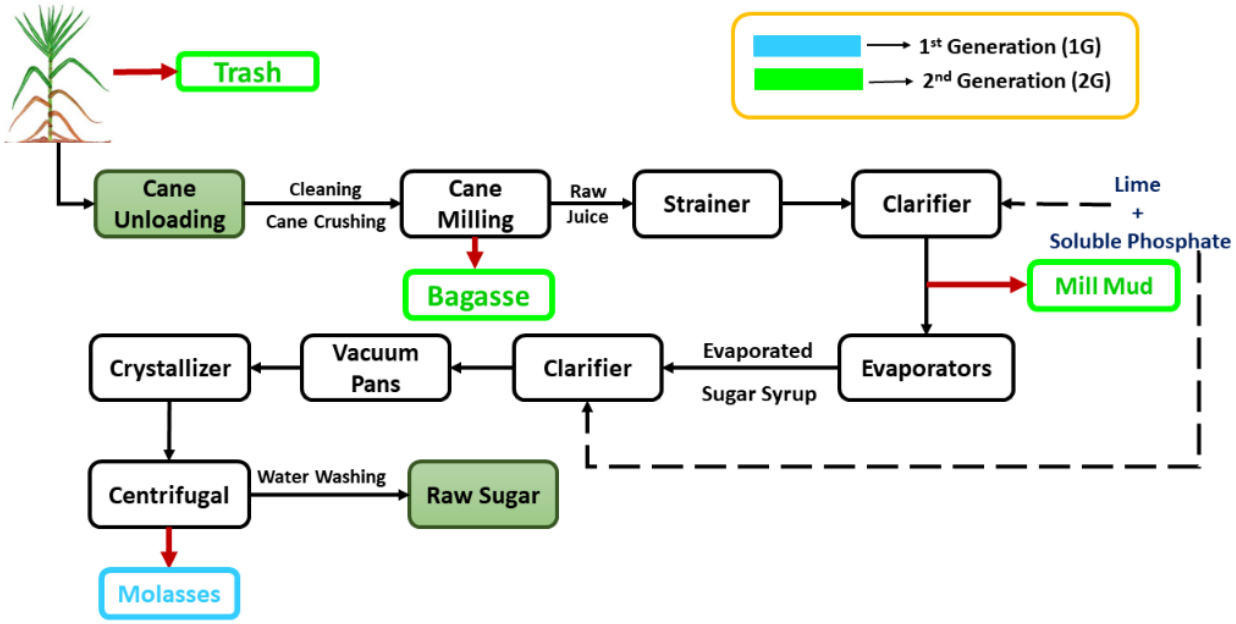

Figure 1. Schematic representation of the basic technological processes carried out in a sugarcane processing industry.

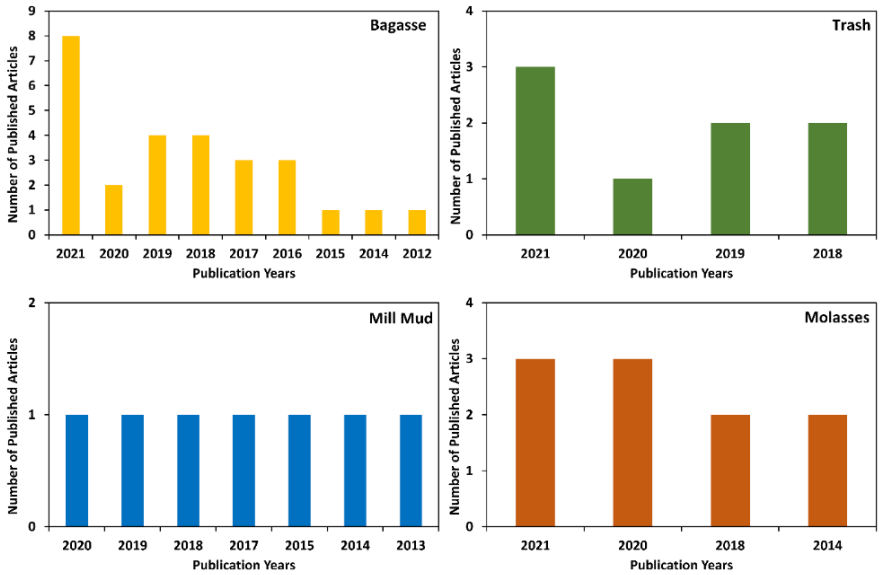

Figure 2. Number of articles published each year (2012–2022) on anaerobic digestion of sugarcane processing industry wastes for methane and hydrogen production (Web of Science Database).

Characterization of 1G and 2G Sugarcane Processing Wastes

All the wastes generated in a sugarcane industry are organic solid wastes, except for molasses, which is a very viscous sugar-rich organic waste. A significant by-product of the sugarcane business that is left in the field following cane harvest is sugarcane dry trash, which is a component of sugarcane tops [7,15]. Bagasse is the fibrous, dry, pulpy residue left behind from the crushing of sugarcane stalks to release their juice. After the juice of sugarcane is filtered, a by-product called mill mud is left behind. It includes bagasse, sugar, and sugarcane soil as well as lime, which is needed in the purification process [16]. Molasses is the viscous sugary residue left after the sugar refinement process takes place. It is dark brown in colour [17]. Table 1 illustrates the approximate chemical composition of the wastes generated because the composition of the wastes may vary due to several factors, like climate, harvesting time, soil nutrients, industrial operating factors, etc.

Table 1. Chemical composition of the 1G and 2G wastes generated in a sugarcane processing industry (TS: Total Solid, VS: Volatile Solid, TKN: Total Kjeldahl Nitrogen, TKP: Total Kjeldahl Phosphorous, FM: Fresh Matter) [15].

Parameter	Units	Sugarcane Trash [15]	Sugarcane Bagasse [15]	Mill Mud [15]	Molasses
TS	% FM	60.87	59.66	27.7	75.97
VS	% FM	54.32	54.67	22.38	68.95
TKN	g/kg	5.78	2.12	4.7	3.71
TKP	g/kg	0.48	0.17	3.1	0.33
C	% TS	41.6	44.6	44.89	37.17
H	% TS	5.8	5.8	6	6.25
O	% TS	52.1	44.5	48.71	38.56
N	% TS	0.45	0.6	0.37	-
S	% TS	0.08	0.1	0.03	-
Cellulose	% TS	30.4	37.7	11.3	ND
Hemicellulose	% TS	18.2	21.7	27.1	ND
Lignin	% TS	27.8	27.3	9.3	ND

From Table 1, it is evident that molasses contains the maximum TS (75.97%) and VS (68.95%) among the wastes generated in a sugarcane processing industry. The carbon content of both bagasse and mill mud is almost the same at 44.6 (TS%) and 44.89 (TS%), respectively, while molasses has the least carbon content at 37.17 (TS%). Bagasse contains

the highest amount of nitrogen and sulphur at 0.6 and 0.1, respectively [15]. All these wastes are generally used for various purposes, which is discussed in the next section.

2. Methane Production via Anaerobic Digestion

The controlled biological breakdown process known as anaerobic digestion (AD) enables the effective production of biogas for various applications, like electricity generation, vehicle fuel, combined heat and power, etc. [18]. A group of bacteria collaborate to carry out AD, converting organic material into biogas and inorganic components. The AD process can be categorized into the following steps:

Hydrolysis:

Enzymes secreted by the microorganisms present in the AD process are used in the hydrolysis reaction to break down complex organic materials, such as carbohydrates, proteins, and fats/oils, into simple monomers and oligomers (sugars, amino acids, and lipids) (extracellular) [19]. The typical reactions (1)–(10) taking place in the hydrolysis stage are as follows:

$$\text{Hemicellulose} + H_2O \rightarrow 2.5\ C_2H_4O_2 \quad (1)$$

$$\text{Hemicellulose} + H_2O \rightarrow C_5H_{10}O_5 \quad (2)$$

$$\text{Cellulose} \rightarrow 2\ C_2H_6O + 2\ CO_2 \quad (3)$$

$$\text{Cellulose} + 2\ H_2O \rightarrow 2\ C_2H_4O_2 + 2\ CO_2 + 4\ H_2 \quad (4)$$

$$\text{Protein} + 0.5\ H_2O \rightarrow 0.75\ C_2H_4O_2 + NH_3 + 0.5\ CO_2 \quad (5)$$

$$\text{Lipid} + 3\ H_2O \rightarrow C_3H_8O_3 + 3\ C_{18}H_{34}O_2 \quad (6)$$

$$2\ C_2H_6O + CO_2 \rightarrow 2\ C_2H_4O_2 + CH_4 \quad (7)$$

$$3\ C_5H_{10}O_5 \rightarrow 5\ C_2H_6O + 5\ CO_2 \quad (8)$$

$$C_5H_{10}O_5 + H_2O \rightarrow 2\ C_2H_4O_2 + CO_2 + 2\ H_2 \quad (9)$$

$$C_2H_6O + H_2O \rightarrow C_2H_4O_2 + 2\ H_2 \quad (10)$$

Acidogenesis:

Acidogenic microorganisms break down the basic monomers and oligomers into short-chain Volatile Fatty Acids (VFAs), carbon dioxide (CO_2), H_2, acetic acid, and other organic molecules. Significant amounts of CO_2 and H_2 are produced during this phase [20]. The typical reactions (11) and (12) taking place in the acidogenesis stage are as follows-

$$\text{Cellulose} + 0.1115\ NH_3 \rightarrow 0.1115\ C_5H_7NO_2 + 0.744\ C_2H_4O_2 + 0.5\ C_3H_6O_2 + 0.4409\ C_4H_8O_2 + 0.6909\ CO_2 + 1.0254\ H_2O \quad (11)$$

$$C_3H_8O_3 + 0.04071\ NH_3 + 0.0291\ CO_2 + 0.0005\ H_2 \rightarrow 0.04071\ C_5H_7NO_2 + 0.94185\ C_3H_6O_2 + 1.09308\ H_2O \quad (12)$$

Acetogenesis:

In this stage, the bacteria directly contribute to the production of methane by converting organic molecules to acetate, CO_2, and H_2 [21]. The typical reactions (13)–(15) taking place in the acetogenesis stage are as follows:

$$C_{18}H_{34}O_2 + 15.2359\ H_2O + 0.482\ CO_2 + 0.1701\ NH_3 \rightarrow 0.1701\ C_5H_7NO_2 + 9.02\ C_2H_4O_2 + 10.0723\ H_2 \quad (13)$$

$$C_3H_6O_2 + 0.06198\ NH_3 + 0.314336\ H_2O \rightarrow 0.06198\ C_5H_7NO_2 + 0.935\ C_2H_4O_2 + 0.6604\ CH_4 + 0.160688\ CO_2 + 0.000552\ H_2 \quad (14)$$

$$C_4H_8O_2 + 0.0653\ NH_3 + 0.8038\ H_2O + 0.0006\ H_2 + 0.5543\ CO_2 \rightarrow 0.0653\ C_5H_7NO_2 + 1.8909\ C_2H_4O_2 + 0.446\ CH_4 \quad (15)$$

Methanogenesis:

This stage involves the two different bacterial types that support the generation of CO_2 and methane from a range of substrates. The slow-growing acetoclastic methanogens group consumes acetate and creates methane. The second group consists of methanogens that use H_2 and create methane while consuming CO_2 [22,23]. The typical reactions (16)–(19) taking place in the methanogenesis stage are as follows:

$$C_2H_4O_2 + 0.022\ NH_3 \rightarrow 0.022\ C_5H_7NO_2 + 1.6\ CH_4 + 0.066\ H_2O + 0.67181\ CO_2 \quad (16)$$

$$14.4976\ H_2 + 3.8334\ CO_2 + 0.0836\ NH_3 \rightarrow 0.0836\ C_5H_7NO_2 + 3.4154\ CH_4 + 7.4996\ H_2O \quad (17)$$

$$C_2H_4O_2 \rightarrow CO_2 + CH_4 \quad (18)$$

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O \quad (19)$$

However, by analysing the generated metabolic products or intermediate products, the metabolic routes of the microorganisms participating in the AD process can be tactfully investigated [24]. All the significant intermediate AD products created during the process, including formate, butyrate, acetate, ethanol, lactate, propionate, and methane, are shown in Figure 3 along with the metabolic pathway that was followed. These products are denoted by the letters (a) to (h).

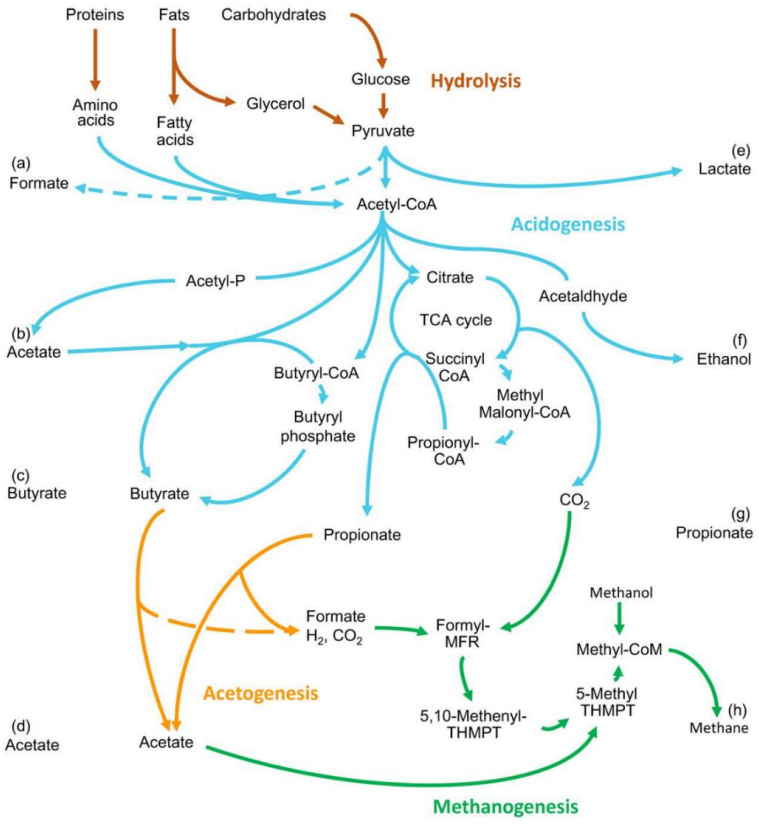

Figure 3. AD Pathway with key intermediates formed (**a**–**h**) [25]. Abbreviations: CoA: coenzyme A; P: phosphate; TCA: tricarboxylic acid; MFR: methanofuran; CoM: coenzyme M; THMPT: tetrahydromethanopterin.

2.1. Factors Effecting Anaerobic Digestion

Anaerobic digestion is a complicated, multi-step process that involves many different kinds of microorganisms, each of which needs particular environmental conditions to survive and perform particular functions. The effectiveness of the digestive processes may be impacted by even the smallest changes in variables. The most important of these factors are covered here.

2.1.1. Organic Loading Rate

The quantity of volatile solids (VS) inserted into the digester on a daily basis is referred to as the "Organic Loading Rate" (OLR) [26,27]. Since methanogen activity is lower during the initial stages than that of the microorganisms that induce hydrolysis or fermentation, a large concentration of organics (high OLR), if frequently provided, will cause to build up VFAs. Up to a definite degree, there might be a linear relationship between rising OLR and the generation of biogas; however, after this point, VFA build up and the ensuing pH drop may prevent bacterial activity [27]. The typical duration of time the substrate spends inside the reactor is known as the hydraulic retention time (HRT). As retention time decreases and reactor volume rises, there is a higher risk of washing out active biomass, which increases capital expenditures [28,29]. The substrate affects the optimal OLR and HRT. González et al. conducted a co-digestion process of sugarcane press mud (2G) with vinasse from ethanol production industry to produce methane. The co-digestion was carried out in a CSTR reactor with an OLR ranging from 0.5 to 2.2 gVS/L/day. However, a partial hindrance of the methanogenic archaea was observed when the OLR was raised to 2.2 gVS/L/day [30]. Ndobeni et al. used upflow anaerobic sludge blanket (UASB) reactor for methane production from sugarcane molasses at 38 °C. An HRT of 48.9 h was used with an OLR ranging from 4.88 gCOD/L/day to 1.86 gCOD/L/day, obtaining a methane yield of 0.71 L/L.day [31].

2.1.2. Temperature

Microbial activity is significantly influenced by temperature. Anaerobic digestion can occur in three operational ranges: psychrophilic (below 20 °C), mesophilic (20–45 °C), and thermophilic (55–70 °C) [18]. Anaerobic digestion microorganisms are highly sensitive to temperature changes that have an immediate impact on the breakdown of organic matter and the creation of methane [19]. Acidogenesis is best in thermophilic ranges (55–70 °C), but it can also stop methanogens from growing. Other advantages of the thermophilic spectrum include early breakdown and a higher organic loading rate. However, the accumulation of fatty acids takes place in the thermophilic range, which has a detrimental impact on methanogenesis and prevents the formation of biogas [18,26,32]. Better process stability and a richer bacterial culture are produced by mesophilic ranges (20–45 °C), although they frequently have low methane generation and degradation rates. Therefore, methanogenesis and hydrolysis function best at mesophilic and thermophilic temperatures, respectively, for anaerobic digestion [32]. From Table 2, it can be concluded that most of the 1G and 2G sugarcane processing wastes' anaerobic digestion was carried out in the mesophilic range. However, Armah et al. conducted an anaerobic co-digestion study of sugarcane bagasse and municipal sludge in batch mode. The authors evaluated the effect of temperature (25, 35, and 55 °C) and OLR (0.5–1.5 gVS/mL) on the process using Box–Behnken design (BBD). The maximum biogas yield of 98.5% was obtained at a minimum OLR of 0.512 gVS/mL and at a lower temperature of 25 °C. The authors concluded that lower temperature is more favourable in the AD process than high temperature for 2G sugarcane waste [33].

2.1.3. Operating pH

The pH scale identifies how basic or acidic an aqueous solution is. The pH of the AD process is one of the main variables regulating bacterial activity [34]. Bicarbonate or carbonate can be added to maintain pH as an alkalinity buffer. Ammonia (Ka = 5.69 1010 at 25 °C) and acetate (Ka = 1.75 105 at 25 °C), which are basic and acidic in nature, respectively,

are examples of natural buffers for anaerobic digestive systems [35,36]. However, it is usually discovered that these buffers are insufficient. Due to the substantial quantity of CO_2 production, the pH can drop [37]. Microorganism development is influenced by pH, and the optimal pH for the AD process is 7.5 [37]. From Table 2, it can be deduced that all the AD processes of 1G and 2G sugarcane processing waste products are carried out at a pH of 7–7.5 for both batch and continuous process. No studies are available on the effect of pH on sugarcane processing wastes. However, Castro-Ramos et al. explored the effect of pH (5.5, 6.5, 7.5, and 8.5) on the cow manure anaerobic digestion process. The production of metabolites was greatly influenced by the initial pH and the length of the fermentation process. At pH 8.5, the most VFAs were produced, and the order of generation of the acids was butyric > acetic > propionic. On days 20 and 4, respectively, the highest synthesis of indole-3-acetic acid and gibberellic acid occurred at initial pH values of 6.5 and 5.5 [38].

2.2. Single-Stage Methane Production from 1G and 2G Sugarcane Processing Industry Wastes

Sugarcane industry wastes consist of both carbohydrates and a small fraction of proteins, which makes them suitable for methane production. Extensive studies have been carried out on these wastes to produce methane. Trash, bagasse, and mill mud are lignin-rich material, and thus an additional stage, i.e., pre-treatment, is essential to produce methane from them. From Table 2, it is evident that most studies have been conducted on these wastes after a pre-treatment was employed. Janke et al. investigated the methane yield of sugarcane straw (Trash) with a pre-treatment process of 12g NaOH/100 g substrate and obtained a maximum methane yield of 231.1 mL/gVS [39]. On the other hand, the same authors conducted AD of ensiled trash and obtained a methane yield of around 250 mL/gVS [40]. Both the studies were carried out under mesophilic conditions and batch process. From the investigations, it can be inferred that chemical pre-treatment and ensiling had an almost similar effect on the methane yield of sugarcane trash. Jafari and Zilouei examined the impact of pre-treating sugarcane bagasse with nano-titanium dioxide (nanoTiO_2) under ultraviolet irradiation (UV), then following it with dilute sulfuric acid hydrolysis. This pre-treatment destroyed the surface morphology of the substrate and also reduced its crystallinity, leading to a methane yield of around 600 mL/gVS via AD [41]. Similarly, Hashemi et al. pre-treated the sugarcane bagasse with ethanolic ammonia at different concentrations. The highest methane yield of 299.3 mL/gVS was observed with 10% v/v aqueous ammonia solution and 50% ethanol at 70 °C for 24 h. The authors claim that these benefits can be attributed to the disintegration of a sizable amount of lignin, alteration of the cellulose's physical characteristics, and increased accessibility of microorganisms to the solid phase's carbohydrates [42]. Srivastava et al. used CuO/Cu_2O-based nano-catalyst prepared from the aqueous extract of the combination of press mud (mill mud) and sugarcane bagasse, which was employed as a reducing agent, for the AD of mill mud. The authors recorded a cumulative methane yield of 224.7 mL/gVS, which was higher than the other batch studies due to the inclusion of copper-based nanoparticles, which, through potential changes in microbial metabolisms, can promote microbial growth, enzyme synthesis, and biogas production [43]. Conversely, González et al. investigated the co-digestion of mill mud and vinasse for the AD process. Methane yields of 365 LCH_4/kgVS and biogas productivities of 1.6 L/L were obtained in co-digestion of mill mud and vinasse (mixing ratio of 25:75), which was 64% higher in comparison to mono-digestion [30]. According to this study, co-digestion of vinasse and mill muck is a viable method for the treatment of streams in the alcohol–sugar business.

Table 2. Single-stage operating conditions and yields of methane production via anaerobic digestion using 1G and 2G sugarcane industry wastes.

Substrate	Co-Digestion	Pre-Treatment	Organism	Reactor/Mode	pH and Temperature	HRT	CH_4 Yield	Kinetic Modelling	References
Trash (2G)	-	Liquid hot water, dilute acid, and KOH solutions	Anaerobic sludge	Batch	pH: 7; T: 37 °C	-	167 L/kg initial TS	Chen and Hashimoto model	[44]
	-	Liquid hot water, dilute acid, and KOH solutions	Solid fraction of anaerobic sludge	Solid-state anaerobic digestion	pH: 7; T: 37 °C	-	214.2 L/kg VS	Modified Gompertz's model	[45]
	-	NaOH solutions	Anaerobic sludge	Batch	T: 38 °C	-	231.1 L/kg VS	Exponential dual-pool two-step model	[39]
	-	Ensiling	Anaerobic sludge	Batch	T: 38 °C	-	Around 250 L/kg VS	Two-step two-pool kinetic model	[40]
	Cow manure	NaOH solutions	Cow rumen	Semi-continuous	-	40/105 days	480 L/kg TVS degraded	-	[46]
	-	-	Anaerobically digested sludge	Batch	pH: 7; T: 37 °C	-	161.8 NmL/gVS	First-order kinetic model	[15]
	-	Steam explosion	Anaerobically digested sludge	Semi-continuous	pH: 7; T: 37 °C	35 days	226 NmL/gVS	-	[15]
Bagasse (2G)	Poultry waste	Thermal	Waste-activated sludge	Batch	-	-	-	Gompertz model, first-order, and logistic model	[37]
	Hemicellulose hydrolysate	Hydrothermal	Anaerobic granular sludge	UASB	T: 20–30 °C	18.4 h	270 L/kg COD	-	[47]
	-	-	Anaerobically digested sludge	Batch	pH: 7; T: 37 °C	-	187.9 NmL/gVS	Modified Gompertz model	[15]
	-	Steam explosion	Anaerobically digested sludge	Semi-continuous	pH: 7; T: 37 °C	35 days	236 NmL/gVS	-	[15]
	-	Acid and enzymatic saccharification	Anaerobically digested sludge	Automatic Methane Potential Test System	pH: 7; T: 37 °C	-	200 NL/kg VS	-	[48]
	Municipal sludge	-	Anaerobically digested sludge	Batch	T: 25, 35, 55 °C	-	-	Modified Gompertz model	[33]
	-	nanoTiO_2 under UV irradiation followed by H_2SO_4 hydrolysis	Anaerobic sludge	Batch	T: 37 °C	-	Around 600 L/kgVS	Modified Gompertz model	[41]
	-	NH_3, Ethanol	Anaerobic sludge	Batch	T: 37 °C	-	299.3 L/kg VS	-	[42]
	Hemicellulose hydrolysate	Autohydrolysis	Acidogenic anaerobic inoculum	Batch	T: 35 °C	-	1.81 Nm^3/Kg TOC	First-order kinetic model	[49]
	Hemicellulose hydrolysate	Autohydrolysis	Acidogenic anaerobic inoculum	Batch	T: 35 °C	-	1.56 Nm^3/Kg TOC	Modified Gompertz model	[50]
	-	-	Bioaugmented cellulose degrading bacteria	Solid-state anaerobic digestion	pH: 7; T: 35 °C	-	440 L/kg VS	Modified Gompertz model	[51]
	-	Hydrothermal		Batch	-	-	197.5 L/kg substrate	-	[52]
Mill Mud (2G)	-	Acidogenic phase of AD	Acidogenic anaerobic inoculum	CSTR	pH: 7.5; T: 37 °C	-	195.5 L/kg VS	First-order kinetic model	[34]
	-	Liquid hot water	Anaerobic sludge	Batch	T: 37.5 °C	-	340.80 L/kg VS	First-order kinetic model	[53]
	-	-			T: 40 °C	-	224.7 L/kg VS	-	[43]
	Trash	-	Anaerobic sludge	CSTR	T: 38 °C	-	Around 250 L/kg VS	First-order kinetic model	[39]
	Vinasse	-	Anaerobic sludge	CSTR	pH:7; T: 35 °C	-	365 L/Kg VS	Hill modified model	[30]
	-	100 °C, Ca $(OH)_2$		Batch	T: 37 °C	-	300 L/kg VS	First-order kinetic model	[54]
Molasses (1G)	-	-	Granular sludge	UASB	T:25–38 °C	48.9 h	0.71 L/L.d	-	[31]

3. Hydrogen Production via Dark Fermentation

Complex organic matter is predominantly broken down and digested during the initial two stages of anaerobic digestion, hydrolysis and acidogenesis, into short-chain fatty acids, alcohols, carbon dioxide, and hydrogen, whose identities and amounts depend on the type of fermentation. This is due to the fact that the microorganisms responsible for the first two stages have a regeneration time of less than 36 h [55]. A substrate made of organic material is transformed into hydrogen, carbon dioxide, and non-gaseous substances like acetic and butyric acids during the acidogenic stage of anaerobic digestion, sometimes referred to as dark fermentation (DF). DF is thought to be a potential alternate plan for generating hydrogen, a pure energy carrier. Dark fermentation is the microbiological method for making hydrogen that is most understood. Anaerobic bacteria break down an organic substance into liquid metabolites (such as tiny molecular acids and ethanol) and generate hydrogen during a process known as dark fermentation [56]. This procedure uses fewer resources and a wider range of basic materials [57]. Hydrogenase is the primary enzyme that controls hydrogen metabolism during dark fermentation [58]. The two fundamental hydrogenases are [FeFe]-hydrogenase and [NiFe]-hydrogenase [59,60]. These enzymes catalyse the reversible reaction (20):

$$2H^+ + 2e^- \leftrightarrow H_2 \quad (20)$$

In contrast to [NiFe]-hydrogenase, which primarily catalyses the oxidation of molecular hydrogen, [FeFe]-hydrogenases are more active in generating molecular hydrogen and are typically sensitive to oxygen [61].

Glycolysis, which involves turning glucose into pyruvate, is the first stage of dark fermentation and a crucial phase in the production of reduced nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) [62]. Pyruvate ferrite oxidoreductase then facilitates the breakdown of pyruvate into acetyl-CoA (acetyl coenzyme A), hydrogen, and carbon dioxide in anaerobic circumstances [61]. Another method is to use pyruvate-formate lyase to catalyse the conversion of pyruvate to acetyl-CoA and formate [63,64]. In the presence of [NiFe]- or [FeFe]-hydrogenase, formate is transformed into hydrogen and carbon dioxide. Additionally, when NADH is simultaneously oxidised and/or adenosine triphosphate (ATP) is created, acetyl-CoA can produce by-products such as acetic acid, butyric acid, and ethanol [65]. Acetic acid, butyric acid, and other metabolites can then be produced from acetyl-CoA. Additionally, butyric acid and ethanol both result in the oxidation of NADH to NAD+, whereas acetic acid does not result in the consumption of NADH. Finally, hydrogenase converts the remaining NADH to hydrogen [61,62]. Figure 4 illustrates the schematic representation of the metabolic pathway for dark fermentation.

The amount of hydrogen ultimately recovered is less than the theoretical maximum stoichiometric amount of 12 mol H_2 per mole of glucose ($C_6H_{12}O_6$) due to the creation of by-products. It can be split into three categories: ethanol fermentation (CH_3CH_2OH), acetic acid fermentation (CH_3COOH), and butyric acid fermentation ($CH_3CH_2CH_2COOH$) [67]. Reactions (21)–(24) illustrate the degrading process.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad (21)$$

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH \quad (22)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CO_2 + 2CH_3COOH + 4H_2 \quad (23)$$

$$C_6H_{12}O_6 \rightarrow 2CO_2 + CH_3CH_2CH_2COOH + 2H_2 \quad (24)$$

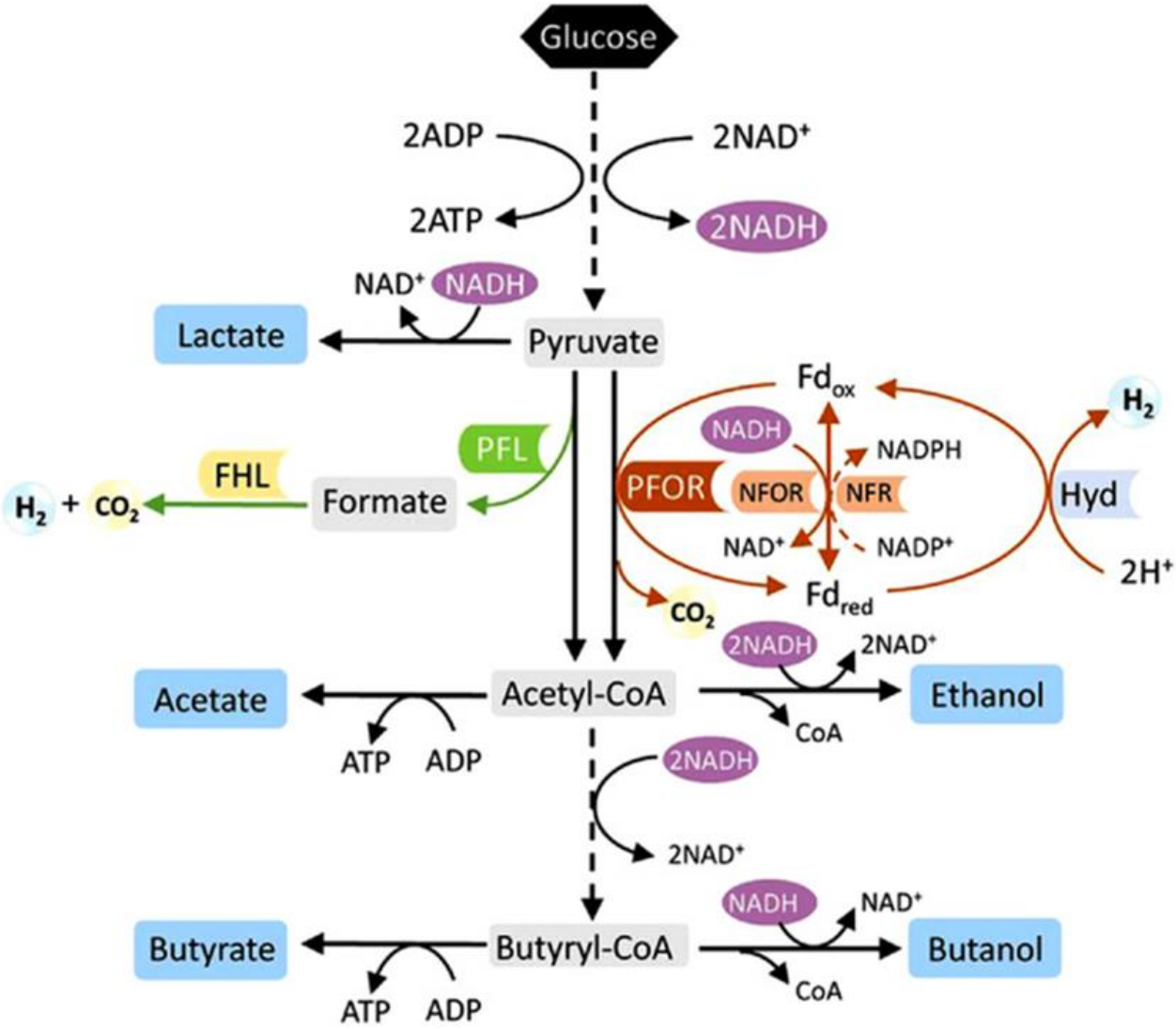

Figure 4. Representative metabolic pathways for hydrogen production during dark fermentation [66]. Abbreviations: PFL: pyruvate formate lyase; PFOR: pyruvate ferredoxin oxidoreductase; NFOR: NADH ferredoxin oxidoreductase; Hyd: ferredoxin-dependent hydrogenase; FHL: formate hydrogen lyase complex; ADP: adenosine diphosphate.

However, the amount of hydrogen created during fermentation is reduced by the development of numerous end products, including acetic acid, propionic acid, and butyric acid, in addition to butanol, methanol, or acetone [68]. Acetic acid production reduces the quantity of molecular hydrogen produced from 12 to 4 moles (Reaction (23)). One mole of glucose only produces two moles of hydrogen if butyric acid is the end result (Reaction (24)). The hydrogen output is further decreased to 1–2.5 mol of hydrogen for every mole of glucose due to the end product's actual composition, which is a mixture of numerous chemical compounds [66]. If 60 to 80 percent of the energy in the substrate is converted to hydrogen, the generation of hydrogen from biomass is seen to be economically viable [69].

3.1. Factors Effecting Dark Fermentative Hydrogen

The yield of fermentation-based hydrogen production might vary depending on a variety of variables. This process' performance and design could be challenging. As a result, various studies on the optimisation of dark fermentation conditions have been conducted in an effort to produce a yield that is close to the theoretical maximum.

3.1.1. Substrates

In dark fermentation, bacteria prefer monosaccharides like glucose, and disaccharides like lactose or sucrose, as carbon sources for metabolic conversions. Starch, cellulose, and hemicellulose are all naturally occurring, renewable sources of sugars that are mostly found in plants as polymers [70]. It is especially practical to use raw materials that are high in starch, which easily hydrolyses to simple carbohydrates. It is more difficult to employ sources that are high in cellulose and hemicellulose [19]. The rigidity of cellulose is due to the beta-1,4-bond that is formed between the glucose monomers, the intermolecular hydrogen bonds between different, parallel cellulose base chains and the fact that these cellulose chains are deeply structured inside cellulose microfibrils makes cellulose very resistant to chemical and biological processes [18]. To alter the chemical composition, extreme conditions are frequently needed, which can dramatically increase processing costs [23]. Jafari and Zilouei used 2G sugarcane bagasse as the substrate for bio-hydrogen and bio-methane production. Due to the high lignin content of the substate, they used nano-titanium dioxide (nanoTiO_2) under ultraviolet irradiation (UV) followed by dilute sulfuric acid hydrolysis to break the crystalline structure of the substate for anaerobic digestion. The highest hydrogen production of 101.5 mL/g vs. (volatile solids) was obtained at 1g nanoTiO_2/L and 120 min UV irradiation followed by 30 min acid hydrolysis of the bagasse [41]. On the other hand, Kumari and Das used alkali pre-treatment of bagasse to enhance the production of hydrogen and methane. This significantly improved bagasse's ability to be broken down by enzymes. In total, 60% of the lignin was completely removed at 0.25 N NaOH concentration (50 °C, 30 min). Alkali-pre-treated sugarcane bagasse boosted the efficiency of enzymatic hydrolysis by around 2.6 times compared to untreated bagasse [71]. On the contrary, Freitas et al. used 1G sugarcane molasses as a substrate for hydrogen production in three expanded granular sludge bed (EGSB) reactors under mesophilic conditions (30 ± 1 °C) [72]. No pre-treatment was required for this substrate, because the carbohydrates present in it are easily accessible to dark fermentative bacteria [73]. Similarly, Li et al. used molasses for hydrogen production, but it was co-digested with *Ginkgo biloba* leaves which increased the hydrogen production from sugarcane molasses by 28.03%. The authors claim that this results from molasses being converted to H_2 by changing the metabolic flux distribution of *E. harbinense* from the ethanol pathway to the acetate pathway [74]. From these studies, it can be concluded that substrate characteristics significantly influence the H_2 production from 1G and 2G sugar mill processing wastes.

3.1.2. Inocula

For dark fermentation, there are two types of inocula: pure and microbial consortia [75]. The most common bacteria for producing dark fermentative hydrogen are *Clostridium*, *Escherichia*, and *Enterobacter*. Manure and sewage sludge are typical inocula utilised in the synthesis of dark fermentative hydrogen [76]. However, they must be prepared before use because there are bacteria that consume hydrogen and reduce the amount of hydrogen produced. Aeration; heat shock; ultrasonication; microwave irradiation; treatment with gamma, ultraviolet, and infrared radiation; and freezing and thawing are physical techniques for inoculum pre-treatment [77]. Acids, bases, and growth inhibitors of microorganisms that consume hydrogen are all used in chemical processes, such as 2-bromoethanesulfonate (BES) and 2-bromoethanesulfonic acid (BESA) [78]. These procedures aim to get rid of bacteria that are sensitive to harsh environments and do not produce endospores, including hydrogentrophs and particularly methanogens [79]. Most of the studies carried out on the production of dark fermentative hydrogen used mixed microbial culture [80,81]. Fuess et al. produced bio-H_2 using mixed culture from 1G sugarcane molasses. The analysis of microbial communities was combined with a thorough temporal and spatial profile of metabolite distribution. The main group responsible for producing bio-H_2 from molasses was the *Thermoanaerobacterium* genus, and it was also determined that the *Caproiciproducens* genus contributed to the manufacture of caproate [82]. Baêta

et al. used acidogenic anaerobic inoculum from a mesophilic pilot plant for producing dark fermentative hydrogen from sugarcane bagasse. The authors utilised a heat treatment (90 °C for 10 min) to get rid of the methanogens that consume hydrogen and enrich the inoculum with microbes that produce hydrogen, like *Clostridium* sp. [50].

3.1.3. Operating PH

Amongst the most crucial operating factors that precisely influence hydrogenase activity, the makeup of the microbial population, the metabolic pathway, and ultimately the breakdown of the substrate, is pH [33]. The creation of organic acids such as acetic, lactic, butyric, and propionic acids during dark fermentative hydrogenation will lower the pH of the fermentation broth and so impede the activity of the enzyme that produces hydrogen [26]. A low pH level also makes it harder for bacteria to keep their cell density at a healthy level. Due to the hydrogen bacteria's considerable activity in the pH range of 5–6.5 in continuously operating reactors, this range must be maintained [80]. The delay time, bacterial growth, enzyme synthesis, and manner of hydrogen production are all considerably impacted by the initial pH values in batch studies [83]. When the starting pH is too low, adverse effects on bacterial growth and hydrogenase synthesis are seen. This resulted in a change to a non-hydrogenic route (more alcohols are created) and prolonged fermentation times [59]. On the other hand, with a higher initial pH value, hydrogenase activity was slowed, and the lag phase was lengthened [84]. As a result, it is critical to keep the pH value within a suitable range for producing dark fermentative hydrogen. From Table 3, it can be observed that all the single-stage bio-hydrogen production from 1G and 2G sugar mill processing wastes, were carried out in a pH range of 5–6.5. All the studies used an acidic ($HCl/H_2SO_4/HNO_3$) or basic (NaOH/KOH) solution to regulate the pH of the substrates and inoculum mixture [57,84–86].

3.1.4. Temperature

Enzyme activity is lowered at temperatures that are either too high or too low [58]. As a result, the ideal fermentation temperature is determined by the type of bacteria present and the substrate being employed. It is thought that substrates that undergo hydrolysis during fermentation respond better to thermophilic and extremely thermophilic environments [87]. The hydrolysis-related enzymes are more active at higher temperatures. Additionally, it is thought that the decreased solubility of gases in water is a contributing factor to the higher yield in thermophilic conditions. A gas at high concentrations can prevent bacterial development [88]. Higher temperatures and mixed cultures, on the other hand, may lead to a depletion of the diversity of bacterial strains and a less thorough breakdown of substrates. This is especially harmful when fermenting wastes and wastewater that are high in organic material from various sources and with different chemical compositions [89]. Additionally, raising the temperature has a favourable impact on the process' kinetics and thermodynamics. The growth and response rates of thermophilic microorganisms are typically faster than those of mesophilic species. The highest quantity of H_2 is produced by a direct conversion of carbohydrates to acetate, which is thermodynamically unfavourable at low temperatures but becomes increasingly so as the temperature rises, making proton reduction to H_2 paired with NADH oxidation exergonic [79]. From Table 3, it can be observed that all the studies were carried out at mesophilic temperatures. Rami et al. produced dark fermentative hydrogen from lactate wastewater at 35 °C and 45 °C. At the end of the fermentation in the reactors that produced hydrogen, bacteria from the *Clostridium* genus predominated at 35 °C, whereas *Sporanaerobacter*, *Clostridium*, and *Pseudomonas* predominated at 45 °C. Because of this, this approach can only work well in mesophilic environments [90].

Table 3. Single-stage operating conditions and yields of dark fermentative hydrogen production from 1G and 2G sugarcane industry wastes.

Substrate	Co-Digestion	Pre-Treatment	Organism	Reactor/Mode	pH and Temperature	HRT	H_2 Yield	Kinetic Modelling	References
Bagasse (2G)	-	nanoTiO_2 under UV irradiation followed H_2SO_4 hydrolysis	Anaerobic sludge	Batch	pH:6.5; T: 37 °C	-	101.5 mL/gVS	Modified Gompertz model	[41]
	Hemicellulose hydrolysate	Autohydrolysis	Acidogenic anaerobic inoculum	Batch	pH:5.5; T: 35 °C	-	0.293 Nm^3/Kg TOC	Modified Gompertz model	[49]
		Alkali	Anaerobic sludge	Batch	pH:6.5; T: 37 °C	-	93.4 mL/gVS	-	[71]
Molasses (1G)	-	-	Mixed culture	Expanded granular sludge bed reactor	pH:4.7; T: 30 °C	1 h	13.92 L/day/L	-	[72]
	-	-	Anaerobic sludge	Anaerobic structured-bed reactor	pH:5.38; T: 55 °C	10 h	8.5 NL/L/d	Monod model	[82]
	Ginkgo biloba	-	*Ethanoligenens harbinense*	Batch	pH:5; T: 37 °C	-	1.58 mol/mol hexose	Modified Gompertz model	[74]

3.1.5. Hydraulic Retention Time

Industrial-scale hydrogen production necessitates the use of continuous and semi-continuous technologies. A major factor determining the hydrogen yield in such systems is hydraulic retention time (HRT) [88]. The average amount of time a substrate spends in a fermentation chamber is measured by hydraulic retention time. The type of substrate utilised in dark fermentation—more precisely, its biodegradability—determines the ideal HRT value. The HRT is often gradually reduced from long to short intervals during continuous culture development to allow for the adaptation of microorganisms to new settings and to prevent the washout of the bacteria of interest [91]. The microbial population dynamically alters because of the HRT shift, causing some species to vanish and others to appear. Methanogens that are slowly forming can be eliminated by combining a brief HRT with continuous stirred tank reactor (CSTR) reactors [92]. Additionally, it permits the use of smaller reactors, which lowers the cost of the apparatus. It is possible to adjust HRT in dark fermentation to decrease or completely eliminate the activity of bacteria using hydrogen in their metabolic processes by taking advantage of fluctuations in the growth rates of hydrogen producers and consumers. The ideal HRT for simple carbohydrates is typically several hours: 2–12 h [91]. Both Freitas et al. and Fuess et al. used sugarcane molasses as the substrate for hydrogen production and used an HRT of 1 h and 10 h, respectively [72,82]. However, Jafari and Zilouei conducted batch studies using sugarcane bagasse for 72–96 h [41].

3.2. Single-Stage Dark Fermentative Hydrogen Production from 1G and 2G Sugarcane Processing Industry Wastes

Biohydrogen can be generated from carbohydrate-rich biomass sources. All the wastes generated from a sugar industry are rich in carbohydrates. However, some of the wastes, like bagasse and trash, need to undergo through a pre-treatment process to make those carbohydrates readily available to the microorganisms. Very rare studies have been carried out on bio-hydrogen production from trash and mill mud, although they are rich in carbohydrates. On the contrary, several studies have been conducted on determining the bio-hydrogen potential of bagasse (2G) and molasses (1G). Table 3 illustrates the different 1G and 2G wastes used for bio-hydrogen production from sugarcane processing industry wastes. Both Jafari and Kumari used sugarcane bagasse as a substrate for dark fermentative hydrogen production in batch process, and the pH and temperature were fixed at 6.5 and 37 °C, respectively [41,71]. However, Jafari produced 101.5 mL/gVS H_2, while Kumari produced 93.4 mL/gVS H_2. This difference in the amount of H_2 produced can be attributed to the different pre-treatment processes carried out by the authors.

4. Challenges and Feasibility of Single-Stage vs. Two-Stage Anaerobic Digestion

Single-stage digesting systems are widely used since they are inexpensive and have a low operational complexity. However, throughout the AD procedure, quickly degradable materials encourage the synthesis of VFAs at rates faster than those absorbed by methanogenic bacteria [28,93]. As a result, the pH decreases, which hinders the process. The use of substances that increase the medium's alkalinity, particular pre-treatment methods, and the implementation of consecutive digesters for segregation of the AD stages are only a few possibilities that can be employed in the prevention or remediation of these issues [94].

Most anaerobic digesters do not provide acidogenic and methanogenic bacteria with the optimal environmental conditions; hence, two-stage digesters adhere to this criterion. Travis was the first to suggest the idea of two-stage AD technology in 1904, and reports on its use and advancement have been documented for many years [57]. The suspended components from the hydrolysed effluent were segregated in a tank with two distinct compartments during the two stages of wastewater treatment used by this researcher. Physical separation of both the acidification and methanogenic stages occurs when two-stage methods are used [95].

With a maximum potential yield of 4 mols of H_2 (acetic acid pathway) for each transformed hexose molecule, molecular hydrogen is a good energy carrier and a gas with a high calorific value (much higher than methane). It is often not feasible to manufacture hydrogen alone with dark fermentation processes due to the significant generation of secondary chemicals and limited hydrogen yield during the sugar conversion process [96]. However, the addition of a subsequent step emphasising the conversion of these molecules into methane utilising the acids produced as by-products from this fermentation may support the procedure and boost the chain's value. Since hydrogen is created and withdrawn for energy purposes, the methanogenic reactor could only produce methane through an acetotrophic process [97]. Reactions (25) through (28) illustrate how sugar is converted into volatile acids and molecular hydrogen.

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H_2 + 4H^+ \quad (25)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 2H_2 + 3H^+ \quad (26)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (27)$$

$$C_3H_6O_3 + H_2 \rightarrow CH_3CH_2COOH + 2H_2O \quad (28)$$

According to Chatterjee and Mazumdar, the advancements and improvements made as a result of the AD phase separation research were created to improve robustness and boost specific methane and hydrogen production [95]. The primary traits of these systems are likewise reported by the same authors as follows:

Single-Stage: Low-complexity production; simple parameter control systems make it easy to operate; less spaces are used; and by combining all the relevant microorganisms in a single reactor, the vital materials are distributed amongst the microorganisms [95].

Two-Stage: These systems enable higher OLRs, allow for condition controls to optimise each group of microorganisms' performance, lessen the shocks brought on by organic loading, and boost the effectiveness of substrate conversion and methane and hydrogen production. The process stability is also excellent for highly fermentable wastes (the hydrolysis phase is not portrayed by them as a limiting step) [95].

Through a comparison of the two approaches, Srisowmeya et al. also outlined the key differences between the two systems (Figure 5), highlighting the decrease in HRTs, the potential for hydrogen production, and the separation of the anaerobic fermentation phases seen in the two-stage system [98].

The benefits of adjusting particular parameters for each of the treatment system's reactors go beyond just the organic load and medium pH. They also apply to other situations [45,99]. The usage of several temperature ranges is intriguing, since methanogenic bacteria do not thrive in single-stage processes because acidogenic microorganisms are highly active under thermophilic conditions and produce substantial amounts of volatile acids [100]. Thus, in the acid reactor, thermophilic temperatures and low pH prevent the growth of methanogenic bacteria, while mesophilic conditions and a neutral pH promote the efficiency and species variety of methanogenic microorganisms in the subsequent stage [97]. Studies on 1G and 2G waste from sugarcane processing industry and its application in two-stage AD processes are discussed in the next section.

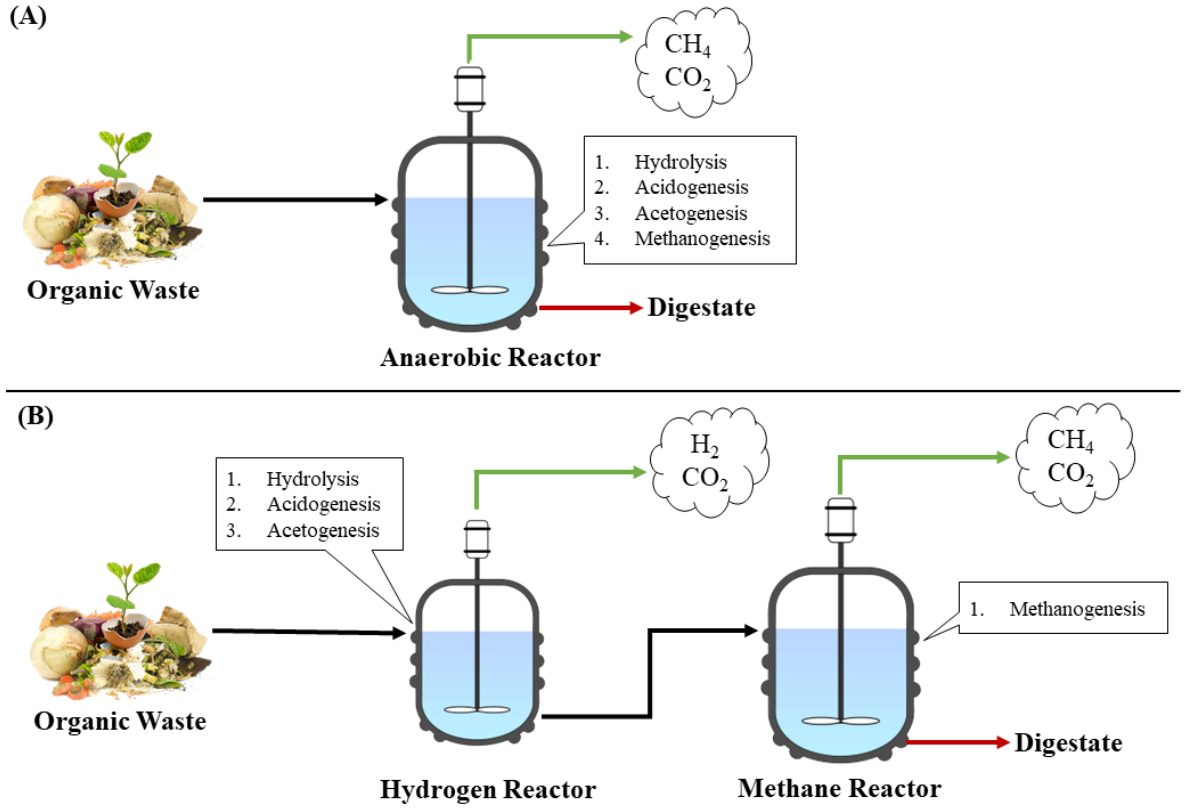

Figure 5. Comparison of (**A**) single-stage and (**B**) two-stage anaerobic digesters.

4.1. Two-Stage Anaerobic Digestion of 1G and 2G Sugarcane Processing Industry Wastes

Very few works have been conducted on all 1G and 2G sugarcane processing industry wastes using two-stage AD processes. Kumari and Das investigated 2G sugarcane bagasse for a two-stage ($BioH_2$ and $BioCH_4$) batch process at 37 °C. The authors chemically pre-treated the bagasse to enhance the enzymatic digestibility of the substrate. The highest hydrogen and methane yield from the treated sugarcane bagasse by two-stage process were 93.4 mL/g-VS and 221.8 mL/g-VS, respectively. The authors concluded that the two-stage procedure led to a significant improvement in energy conversion efficiency of 44.8% as compared to a single-stage hydrogen production process (5.4%) [71]. Conversely, the same authors conducted reactor studies with sugarcane bagasse for $BioH_2$ and $BioCH_4$ production. To lessen the usage of expensive chemicals in the fermentation process, the authors combined bagasse and water hyacinth. The authors used a continuous stirred tank bioreactor to produce biohythane in two stages using a mixture of pre-treated sugarcane bagasse and water hyacinth (1:2 ratio; soluble COD: 302 g/L). The authors concluded that 8 h and 10 days, respectively, were the ideal HRT for the synthesis of $BioH_2$ and $BioCH_4$, which resulted in maximum yields of 303 mL/g COD and 142 mL/g COD for H_2 and CH_4, respectively. The greatest gaseous energy recovery from the continuous biohythane manufacturing process was 8.97 kJ/g COD, increasing substrate conversion efficiency overall by up to 86% [101].

On the contrary, Vilela et al. used 1G sugarcane molasses in a two-stage continuous thermophilic (55 °C) system to produce $BioH_2$ and $BioCH_4$. A high OLR of 120 $KgCOD/m^3/day$ and HRT of 2 h was maintained by the authors for the acidogenic ($BioH_2$) reactor, while the methanogenic ($BioCH_4$) reactor was varied from 1–25.2 $KgCOD/m^3/day$ and the HRT was at 240 h. The authors witnessed around 1700 NmL/L/day of H_2 production and 350 NmL/L/day of CH_4 production at low OLR of 1–2.3 $KgCOD/m^3/day$ [102]. The authors also carried out a thorough metabolite and molecular analysis, from which they deduced that the syntrophic and acetoclastic activities (accumulation of acetate, propionate,

and lactate) were ineffective and that the hydrogenotrophic methanogenesis, specifically by the *Methanothermobacter* genus, was the predominant methane-producing pathway in CH_4. Last but not least, the energetic potential of molasses (8560 kJ/kg$COD_{applied}$) surpassed vinasse's by at least 25%, showing that the significant availability of biodegradable organic matter in molasses necessitates a low OLR to provide effective bioenergy recovery levels [102]. Similarly, Oliveira et al. conducted a comparison study of two-stage and single-stage thermophilic (55 °C) anaerobic systems for H_2 and CH_4 production from sugarcane molasses. The two-stage (3912 NmL/L/day) process showed 1.5 times higher CH_4 production than a single-stage process (2688 NmL/L/day), and 200% higher bioenergy was obtained from the two-stage compared to the single-stage process [103].

4.2. Energy Balance of Single-Stage vs. Two-Stage Anaerobic Digestion Process Using 1G and 2G Sugarcane Processing Industry Wastes

Energy assessments of both 1G and 2G sugarcane processing industry wastes were carried out for single-stage and two-stage anaerobic digestion processes. This was calculated using the lower calorific value of hydrogen (241.8 kJ/mol H_2) and methane (802.6 kJ/mol CH_4) [104]. Table 4 represents the energy yield obtained from different studies.

Table 4. Energy yield of 1G and 2G sugarcane processing industry wastes.

Substrate	First-Stage	H_2 Energy Yield	Second Stage	CH_4 Energy Yield	Total Energy Yield	Reference
Trash (2G)	-	-	291 mLCH_4/gVS	10.42 KJ/gVS	10.42 KJ/gVS	[39]
	-	-	Around 250 mLCH_4/gVS	Around 8.95 KJ/gVS	8.95 KJ/gVS	[40]
Bagasse (2G)	101.5 mLH_2/gVS	1.09 KJ/gVS	-	-	1.09 KJ/gVS	[41]
	93.4 mLH_2/gVS	1.008 KJ/gVS	-	-	1.008 KJ/gVS	[71]
	-	-	Around 600 mLCH_4/gVS	Around 21.49 KJ/gVS	21.49 KJ/gVS	[41]
	-	-	299.3 mLCH_4/gVS	10.72 KJ/gVS	10.72 KJ/gVS	[42]
Mill Mud (2G)	-	-	195.5 mLCH_4/gVS	7.004 KJ/gVS	7.004 KJ/gVS	[34]
Molasses (1G)	2.8 LH_2/L-reactor/Day	30.225 KJ/L-reactor/Day	1.48 LCH_4/L-reactor/Day	53.02 KJ/L-reactor/Day	83.245 KJ/L-reactor/Day	[105]
	4.56 LH_2/Day/L	49.2 KJ/Day/L	-	-	49.2 KJ/Day/L	[72]

From Table 4, it can be inferred that the energy obtained from two-stage AD processes is significantly greater than single-stage processes. However, the difference in energy yields from the 2G substrates are due to the different pre-treatment methods adopted by the authors. Apart from the mentioned 1G and 2G wastes, there are some intermediate substrates produced in sugarcane processing industry which can be used for two-stage AD processes, like crushed cane (2G) and sugar syrup (1G). Very few studies have been carried out using these wastes [106,107].

5. Conclusions

Anaerobic digestion is a promising technology which can be exploited to produce both H_2 and CH_4 from organic substrates. From this review, it can be concluded that very few studies have been published on 2G sugarcane processing industry wastes. Moreover, two-stage studies on both 1G and 2G wastes have not been evaluated in detail. From the above review, it can be demonstrated that almost all of the 1G sugarcane industry wastes require a certain amount of pre-treatment, like steam explosion, hydrothermal, ensiling, chemical treatment, etc., before the AD process. Many reactor configurations have ideal conditions and needs to promote the growth of the microorganisms participating in the process and maximise their production. Using a two-phase reactor system using 1G and 2G wastes, it is possible to make hydrogen and methane from AD at the same time. However, to produce H_2 and CH_4 from 2G sugarcane processing industry wastes will require pre-treatment, which will increase the operating costs, but an optimum AD process can economically benefit the entire operation. To summarize the findings from this

review, it can be concluded that wider research can be carried out using these substrates as single-stage and two-stage AD processes.

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