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Review

Exploring Potentials for Bioresource and Bioenergy Recovery from Vinasse, the “New” Protagonist in Brazilian Sugarcane Biorefineries

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Abstract: Vinasse management in biorefineries bears a burden for sugarcane industries. Despite its high potassium-related fertilizer potential, a series of negative environmental impacts is expected to occur in long-term soil applications of vinasse through fertirrigation. Conversely, a high biodegradable organic content characterizes vinasse as a potential substrate for bioresource and bioenergy recovery from numerous (bio)technological perspectives. This review presents the alternative approaches proposed for sugarcane vinasse management in Brazil, with special attention dedicated to the role of anaerobic digestion as the core conversion step. The suitability of applying phase separation, i.e., the separation of fermentation from methanogenesis in sequential reactors, is discussed in detail. Laboratory and full-scale experiences were considered to discuss the energetic potential of sugarcane vinasse through biogas generation. With a national installed capacity of up to 1603 MW, energy from vinasse could replace half of the coal-derived electricity in Brazil. Meanwhile, investing in vinasse fermentation to obtain soluble organic metabolites could provide more than 10 g L^{-1} of (iso)butyrate. This is the first review addressing the potential use of sugarcane vinasse in anaerobic biorefineries that discusses applications far beyond conventional biogas production, and encourages the rational use of vinasse as a raw material for bioprocesses, either in short- or long-term scenarios.

Keywords: sugarcane biorefinery; vinasse management/exploitation; biodigestion; two-phase systems; bioresource production; bioenergy recovery

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1. Residual Stream Management in Sugarcane Biorefineries

Sugarcane-based sugar and ethanol plants are representative examples of the biorefining concept based on the potential production of a wide range of value-added products from sugarcane, primarily sugar and ethanol [1]. The bagasse resulting from cane crushing is burnt in cogeneration plants for the production of both electricity and steam, which directly supply various processes and operations of the plants (Figure 1a). Steam is fully consumed in specific steps of sugarcane processing, such as juice evaporation (sugar production) and wine distillation (ethanol production). Meanwhile, only a fraction of the produced electricity is consumed within the boundaries of the biorefinery, and the remaining (surplus) amount is exported to the grid [2,3]. This characterizes electricity as a third major product of sugarcane biorefineries. Junqueira et al. [4] indicated a potential surplus electricity amount of 186 kWh per ton of cane (TC) in optimized first-generation (1G) biorefineries, which are characterized by a series of technological improvements compared to conventional industrial schemes, such as recovering trash from the fields, using molecular sieves instead of azeotropic distillation, and efficient heat integration within the whole plant to reduce steam consumption.

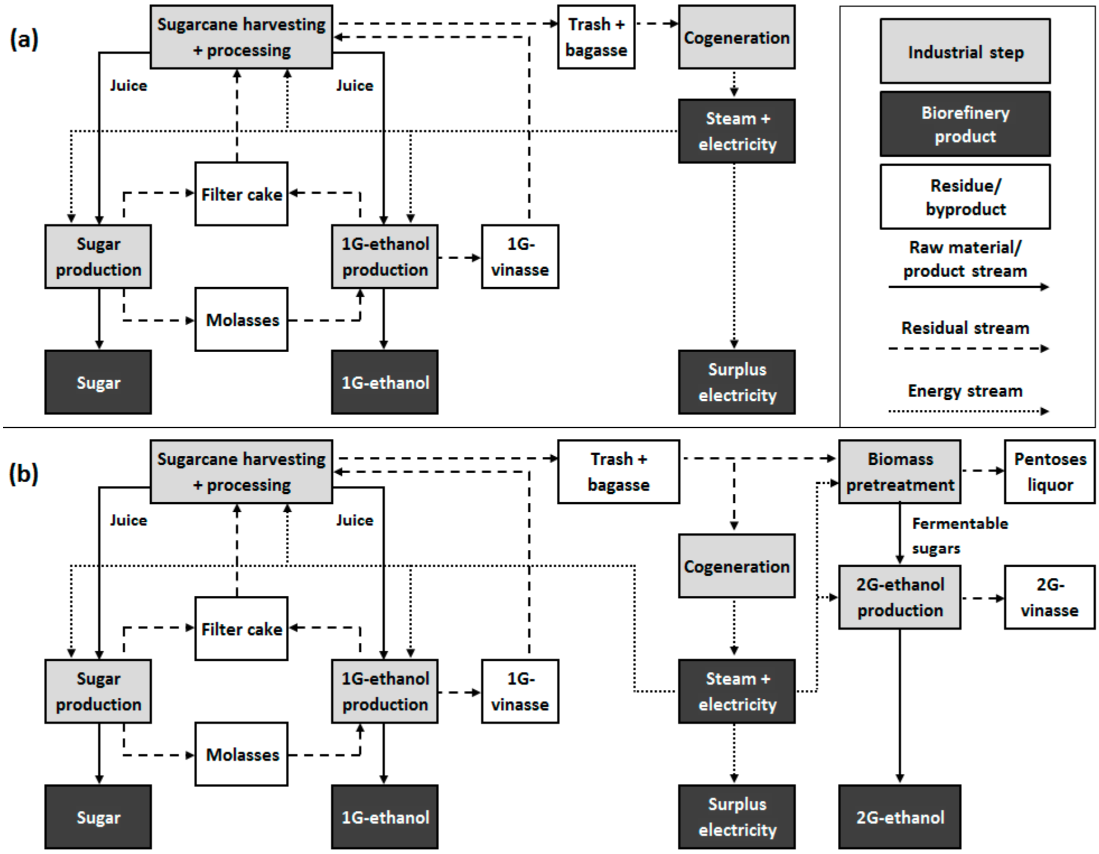

Figure 1. Generation and destination of solid and liquid residual streams in (**a**) conventional first generation (1G) and (**b**) integrated first and second generation (1G2G) sugarcane biorefineries.

With respect to residual stream management from sugarcane biorefineries, two different scenarios can be observed when considering solid and liquid fractions. Relatively well-defined strategies are established for the solid residues, while some uncertain approaches are still observed in the case of wastewaters. Two primary destinations are virtually defined for solid residues from sugarcane processing: [i] the thermoelectric generation from bagasse in the short- to medium-term, as aforementioned, and [ii] the integration of second-generation (2G) ethanol production in the long-term (Figure 1b) [5–8]. 2G processes aim to increase the ethanol productivity in biorefineries by converting lignocellulosic materials via biochemical or thermochemical processes. The first ones are based on the fermentation of sugars obtained after pretreating lignocellulosics (Figure 1b), while the latter use the gasification of the residues [3,7,9]. Recent studies also propose the biodigestion of lignocellulosics for biogas production [8,10–13] as an alternative route for enhanced bioenergy generation. In addition to bagasse and trash, filter cake is another important solid residue from biorefineries. This nutrient-rich material is generated specifically in the treatment of juice (before the production of sugar and ethanol), and also presents a defined destination (together with trash) as a soil conditioning agent [14,15].

Conversely, the management of residual liquid streams in biorefineries, especially considering vinasse, may still be considered a burden for the sugar and ethanol industry. Vinasse is characterized as a high-strength wastewater (HSW) resulting from distillation in ethanol plants, and it presents a high content of biodegradable organic matter, macro- and micronutrients, and solids [16–18]. Fertirrigation is virtually the only management approach for vinasse in sugarcane biorefineries and is characterized by the direct land disposal of the effluent into sugarcane fields for recycling nutrients and water in crops [3,18,19]. Although fertirrigation leads to significant reductions in terms of mineral fertilizer use, notably potassium and phosphorus, many negative environmental impacts should be observed in continuous long-term vinasse applications, such as soil salinization, soil/water acidification, and an increase in greenhouse gas (GHG) emissions [17,18,20–22]. Drawbacks in terms of wastewater management in sugarcane biorefineries may become even more relevant in the future, because new sources of residual streams from 2G processes will be made available, which include pentose liquor (and eventually deacetylation liquors from trash processing [8]) and 2G-vinasse (Figure 1b). The pentose liquor is generated during the pretreatment of lignocellulosic residues, while 2G-vinasse results from 2G-ethanol distillation. Although little is known in terms of the compositional aspects of these HSW, high concentrations of toxic compounds may be found in the pentose liquor, such as furfural and 5-hydroxymetilfurfural, while 2G-vinasse is poor in terms of nutrients [3,23].

When focusing on 1G sugarcane distilleries, numerous treatment approaches have been proposed in an effort to reduce the polluting load of vinasse. These include conventional coagulation–flocculation [24–26] and adsorption processes [27], advanced electrochemical and oxidative processes [25,26,28–30], and conventional aerobic [31,32] and anaerobic processes [33–40]. Although these approaches are environmentally relevant, from the biorefinery perspective, the treatment processes should add value to residual fractions instead of only being a step to reduce environmental impacts [41–43]. In this context, the high biodegradable organic content (e.g., organic acids, residual ethanol, carbohydrates, and glycerol) usually found in sugarcane vinasse, i.e., biochemical oxygen demand (BOD) values ranging from 11.3–29.7 g L^{-1} [32,44–47], is a potential substrate for a series of biotechnological applications, including the recovery of energy through optimized anaerobic processes [3,48–53] and the production of value-added products, such as enzymes and biosurfactants [54,55]. The high nutrient content found in vinasse may also be properly exploited in alternative bioprocesses, such as the growth of microalgae and protein-rich microorganisms [56–58].

Anaerobic digestion, or simply biodigestion, is the most attractive technological approach for managing sugarcane vinasse within the context of biorefineries because the environmental suitability of the effluent (resulting from a decreasing polluting load) may be coupled to the production of a wide range of bioresources and/or biogas. Biogas production characterizes the primary approach for the exploitation of HSW through biodigestion, as observed in experimental (lab and pilot scale) schemes [33,35–38,40,59,60] and theoretical estimations [48–50,61–66] proposed for sugarcane vinasse. In particular, using phase separation as an operating strategy for anaerobic systems, in which fermentation (first phase) is separated from methanogenesis (second phase), considerably extends the range of options for the recovery of bioresources from organic waste [41–43,67,68]. The fermentative (or acidogenic) step can be exploited to produce numerous metabolites other than methane-rich biogas, such as biohydrogen (bioH_2), organic acids, solvents, and biopolymers. An even more efficient use of the primary raw material, i.e., sugarcane, can be observed for this case, with a view to adopting the cascade approach [41,69]. This concept is based on the application of sequential processes to directly and/or indirectly (i.e., through the production of bioproducts, such as sugar and ethanol) recover energy from a central energy flow, which represents the energetic potential of the raw material.

This review aims to discuss the potentials of sugarcane vinasse as a substrate for bioresource and bioenergy recovery from different (bio)technological routes, using the concept of biorefinery as the reference. The compositional aspects of sugarcane vinasse are first presented, especially considering the biodegradable organic fraction and nutrient content. Different applications for vinasse are then considered, such as using it as a carbon and nutrient source for growing microorganisms and producing organomineral fertilizers. Special attention is given to the role of biodigestion as the central process for efficiently exploiting vinasse, while considering the state of the art of conventional biodigestion schemes and proposing layouts with phase separation for diversifying the recovery of resources in sugarcane biorefineries. Focus is given to the Brazilian case, which is characterized by the second largest world ethanol production, in which vinasse mostly results from the conversion of blends of sugarcane juice and molasses in biorefineries, due to the integration of distilleries and sugar plants. This review describes the fate of sugarcane vinasse based on the biorefinering concept, i.e., proposing the maximum exploitability of this energy-rich substrate.

2. Sugarcane Vinasse: Biodegradable Organic Fraction and Nutrients

The composition of vinasses from various feedstocks is well characterized in the reference literature, as observed in studies published throughout the last decades, which include España-Gamboa et al. [16], Fuess and Garcia [17], Wilkie et al. [23], Christofoletti et al. [70], and Sheehan and Greenfield [71]. Therefore, this section focuses specifically on the characterization of the biodegradable organic fraction and nutrient content of sugarcane vinasse, because the technological application of this HSW largely depends on these aspects. Table S1 shows the compositional characteristics obtained for sugarcane vinasses in various studies, in which both organic and inorganic fractions are described in detail. The high biodegradable content found in sugarcane vinasse is observed based on specific parameters, such as the BOD/COD ratio (values usually above 0.40), the concentration of total carbohydrates (up to 16.3 g L^{-1}), and the concentration of organic acids—primarily lactic (up to 12,697 mg L^{-1}), iso-butyric (1547–4597 mg L^{-1}), and malic (2639–10,460 mg L^{-1}) acids (Table S1).

The biodegradable organic fraction found in vinasse results from low molecular weight organic compounds that are separated from ethanol in the distillation process [72,73]. These compounds result from three major sources: residual concentrations of non-fermented carbohydrates, residual concentrations of non-recovered ethanol, and metabolites from parallel conversion (fermentative) pathways carried out by yeasts and bacteria. The proportion of organic components in vinasse mainly depends on the conditions used in the fermentation step, such as the concentration of reducing sugars, pH, and microbial control. The presence of organic metabolites in sugarcane vinasse usually accounts for a large fraction of the biodegradable organic content of the effluent (Table S1), so that the presence of fatty acids, mainly lactic, results directly from the contamination of fermentation vessels by bacteria. Glycerol also comprises a highly relevant metabolite found in vinasses and is characterized as the main byproduct released by yeasts subjected to stress conditions [73–75]. Approximately 2–9% of the total reducing sugars available to yeasts are converted into glycerol, leading to concentrations in the range of 1.4–5.9 g L^{-1} [43,72,75–81]. Comparatively, the production of acetic acid in parallel metabolic pathways should be minimal, accounting for only 0.1–0.7% of the available reducing sugars [74]. In turn, residual ethanol concentrations vary considerably in sugarcane vinasse, with the observation of low (250 mg L^{-1} [82]) and very high (3.8 g L^{-1} [72], 5.5 g L^{-1} [77]) values. Ethanol concentrations in vinasses resulting from spirit production can reach even higher values (15.8 g L^{-1} [78]), because both the head (55–65% v/v of ethanol) and the tail (<38% v/v of ethanol) of the distilled product are discarded together with vinasse [83]. The terms "head" and "tail" refer to the liquid fractions recovered respectively at the beginning and at the end of the distillation step, representing 5–10% (head) and 10–15% (tail) of the total distilled volume [83]. The head usually contains relatively high concentrations of methanol and aldehydes, while the ethanol fraction in the tail is below the desirable content (43–45% v/v) found in the spirit. Hence, only the "heart", i.e., the amount of liquid collected between the head and the tail, effectively corresponds to the spirit (75–80% of the total distilled volume) [83].

With respect to the nutrient content, vinasses resulting from sugarcane processing are characterized primarily by high concentrations of potassium and sulfate (Table S1). Potassium comprises one of the most abundant nutrients in vegetable tissues [84] and is incorporated into the ethanol production chain when sugarcane is crushed to extract the juice. In turn, high levels of sulfate result from using sulfur compounds in specific steps of sugar and ethanol production. In particular, high levels of sulfuric acid are dosed during the fermentation to both prevent the bacterial contamination and the flocculation of yeast through pH control [85,86], which directly favors the buildup of sulfate in vinasse [48,79,87]. The presence of other compounds, such as nitrogen and phosphorus, may also be explained by their incorporation from plant tissues, as well as by using chemicals in sugarcane harvesting and processing [17,85].

3. (Bio)Technological Applications (Other Than Biodigestion) for Sugarcane Vinasse

The agricultural use of sugarcane vinasse in fertirrigation has historically limited the study of alternative approaches for its management. In practical aspects, fertirrigation is still considered the most suitable techno-economic approach (by decision-makers in the sector) for vinasse in the Brazilian sugar and ethanol industry. In fact, fertirrigation has virtually been the only management approach for sugarcane vinasse in Brazil since the mid-1970s [3], which has led to numerous studies (targeting mainly a local audience) on the effects of vinasse for soil and sugarcane crops. These studies include Fuess and Garcia [17], Fuess et al. [18], Fuess et al. [88], Brito et al. [89], Brito et al. [90], Camilotti et al. [91], Gunkel et al. [92], Lyra et al. [93], Miranda et al. [94], Moore et al. [95], Ribeiro et al. [96], Rolim et al. [97], Silva et al. [98], Tasso Jr. et al. [99], Uyeda et al. [100], and Zolin et al. [101]. Despite the scientific relevance of these studies, this scenario reinforces the importance of developing options that enable more efficient exploitation of this HSW as a raw material in diversified processes.

The reference literature indicates relatively few biotechnological alternatives in which sugarcane vinasse was used as the raw material, as compiled in Table S2, which highlights the use of its biodegradable organic and nutrient contents as substrate in bioprocesses dedicated to the cultivation of microorganisms and/or the production of biochemicals. In this case, some examples based on using sugarcane vinasse obtained directly from molasses fermentation were also considered. The growth of microalgae on sugarcane vinasse was studied by Ramirez et al. [57], Bonini [76], Kadioğlu and Algur [102], Marques et al. [103], Santos et al. [104], Candido and Lombardi [105], and Soto et al. [106], while Nitayavardhana and Khanal [56], Pires et al. [107], Ricci et al. [108], and Santos et al. [109] used vinasse as a nutrient source in the cultures of protein-rich fungal biomass (Table S2). Most of these studies have two primary motivations: the reduction in the polluting load of vinasse and the minimization of production costs of the target products, such as single-cell protein [108] by using low-cost substrates. Despite the successful application of sugarcane vinasse as a nutrient source, diluting the effluent is a common characteristic in most of the considered studies [56,57,103,104,107]. This strategy may lead to a major drawback in full-scale applications, due to the high specific vinasse generation rate (12–14 m^3 per m^3 of ethanol) [17,19,64], which directly requires huge processing units. Alternatively, vinasse clarification using proper coagulants can eliminate dilution requirements while maintaining adequate nutrient concentrations in the cultivation broth [110].

With respect to the recovery of biochemicals, Aguiar et al. [54] and Kahraman and Gurdal [111] assessed the role of vinasse as a nutrient source to produce high-value enzymes (laccase and peroxidases) from fungi, while Oliveira and Garcia-Cruz [55] and Colin et al. [112] studied the recovery of natural emulsifiers (Table S2). Bastos et al. [113] also assessed the potential to recover citric acid from bagasse by using vinasse as a moistening agent. Similarly to studies focusing on biomass production, vinasse dilution is characterized as an imperative factor for achieving successful results in such cases [111,112,114]. Moreover, using pure cultures, as well as processes based on microbial biomass production, usually requires the sterilization of wastewater streams, which also limits full-scale applications. Vinasse naturally carries yeast and bacteria from the fermentation vessels. It is important to stress that studies on the recovery of biochemicals from sugarcane vinasse are relatively scarce compared to exploiting vinasses from different feedstocks, as observed from surveys in the specific literature. Vinasses resulting from the processing of corn, wheat, grains, grape and rice (also known as thin stillages for the cases of corn, wheat, and grains) have already been assessed as raw material to produce a wide range of bioproducts, such as: acid protease [115], polysaccharides (10,000–200,000 Da) [116], eicosapentaenoic acid [117], lipids (single-cell oils) [118], bacterial cellulose [119,120], 1,3-propanediol [121], carbon-based solid acid catalyst [122], lactic acid coupled to animal feed [123], cellulosic fibers for 2G-ethanol production [124], and chitosan [125]. This discrepancy in terms of diversified studies also results from the massive land disposal of sugarcane vinasse via fertirrigation, which characterizes a limiting factor for enhancing the exploitation of sugarcane vinasse using alternative processes.

In particular, recycling vinasse in fermentation to produce fuel ethanol and spirits has been demonstrated to be an attractive approach for the recovery of biochemicals in sugarcane biorefineries, as proposed by Navarro et al. [73], Menezes et al. [114], Pereira [126], and Madaleno et al. [127] (Table S2). Using optimized operating conditions (which include recycling yeasts), Navarro et al. [73] obtained high ethanol concentrations (>70 g L^{-1}) throughout 14 cycles meant to reduce the inputs of nutrients, fresh water, and sulfuric acid in fermentation vessels. Moreover, vinasse recycling characterized a bioconcentration step, which could reduce the energy consumption in further concentration steps using conventional evaporators [73]. Regarding different feedstocks, recycling thin stillages in corn- and cassava-to-ethanol industries has been widely studied [128–131], and results also demonstrated the technical feasibility of this approach. Bialas et al. [128] highlighted that up to 75% of the fresh water used in the fermentation step could be replaced by stillage (subjected only to simple steps of cooling down and centrifugation prior to recycling), while the energy consumption using stillage concentrations in multi-evaporator processes could be considerably reduced [131]. Therefore, among the technological approaches for the enhanced recovery of biochemicals in sugarcane biorefineries, vinasse (fresh or biodigested) recycling for enhanced ethanol production could be a wise choice, based primarily on a more efficient energy extraction from the biodegradable organic fraction of vinasse.

Additional alternative approaches to sugarcane vinasse management reported in the literature include the production of organomineral fertilizers [132,133], the use in nutritive solutions for hydroponics [134], the generation of energy from burning [135,136], and the use as a biostimulating agent for the biodegradation of hydrocarbons and oily residues [137,138] (Table S2). A recent highly innovative application was described by Tamashiro et al. [139], in which vinasse replaced water in concrete manufacturing, together with recycled concrete aggregates as substitutes to sand. The production of organomineral fertilizer may also be considered an attractive option for vinasse management in full-scale biorefineries, which works primarily by facilitating the transportation and field applications of vinasse, as well as by improving some physical-chemical properties of the vinasse due to incorporating solid residues [140]. Adding different residues, namely grape-marc, olive-pressed cake, and cotton gin trash to beet molasses vinasses improved the nutrient release in previous reports [141–143], and therefore a similar behavior should be observed for sugarcane vinasse. However, when considering biorefineries, unlike the scheme proposed by Gurgel et al. [132] (Table S2), in which raw vinasse is concentrated and blended with other residual streams from biorefineries, the biodigestion of vinasse (prior to the concentration step) could improve the energetics of the process, as proposed by Nandy et al. [133] in the Indian ethanol industry. Concentration is a highly energy-consuming step, which may seriously affect the economic feasibility of full-scale systems [47,144]. Moreover, condensed liquids recovered from raw vinasses are still characterized by high acidity [145] due to the volatilization of organic acids, which requires additional treatment steps to enable proper reuse, either as process water or for return to the sugarcane fields. Peiter et al. [146] pointed out the sequential application of anaerobic digestion and reverse osmosis as the most attractive approach to manage vinasse on an exergy basis; however, the economic performance of evaporators outperformed that from reverse osmosis.

The high energy inputs associated with the concentration step were also considered a major drawback in the system proposed by Cortez and Pérez [136], which was characterized by the combustion of concentrated vinasse blended with fuel oil (Table S2). Additional drawbacks reported by these authors included negative effects from increasing vinasse concentrations over burning oil, as a direct result of the emulsification of the hydrocarbon, as well as the fusion of ashes within the bed combustor. Alternatively, Akram et al. [135] co-fired raw sugarcane vinasse with coal, and also reported operating problems due to the agglomeration of ashes within the combustor (Table S2). In addition to the unsuccessful experiences with vinasse burning, using complementary fossil energy sources associates negative environmental impacts with this approach, such as the depletion of fossil resources and the release of GHG. The potential recovery of potassium-rich ashes from combustors, which present high agricultural value (similar to that of organomineral fertilizers), represents one of the few benefits of this approach [47,133].

In summary, the large-scale application of most of the technological alternatives evaluated for the management of sugarcane vinasse still demands process improvements while considering important limitations, such as diluting the vinasse and nutritional supplementation to optimize the recovery of bioresources. Moreover, little is reported on the physical-chemical characterization of vinasse after (bio)processing, which further limits understanding of the potential of these approaches in full-scale plants. Among the listed alternatives, recycling vinasse as a complementary carbon and nutrient source for the fermentation step and organomineral fertilizer production may be characterized as important alternatives, as minor modifications would be required in the operating plants. However, the recovery of bioresources or bioenergy from vinasse in sugarcane biorefineries could be maximized by using biodigestion, particularly with phase separation.

4. Biodigestion as the Core Technology for Processing Sugarcane Vinasse

4.1. Fundamentals of Anaerobic Processes

The reference literature historically compiles a series of critical studies on the anaerobic digestion of wastewater, which range from fundamental and general aspects [147–152] to specific studies dealing with different types of high-rate systems [153–156] and using biodigestion for vinasses [23,157]. Biodigestion is a natural biological process in which the organic matter is converted, when there is a lack of free oxygen, by diverse groups of microorganisms that are syntrophically associated. The primary product of the process is biogas, a gaseous blend mainly consisting of methane (CH_4) and carbon dioxide (CO_2). Using biodigestion as an approach to treat organic residues has been reported for several decades. However, a significant development in the process was observed only from the 1970s onwards, when the search for alternative energy sources stimulated a significant technological improvement of anaerobic systems. The development of high-rate reactors was *sine qua non* for such progress [149,153,155]. High-rate reactors triggered significant improvements in the anaerobic conversion of HSW by decoupling the solid retention time (SRT; or sludge age) from the hydraulic retention time (HRT) in order to provide adequate contact periods for microorganisms, particularly methanogens, to degrade organic compounds [149,155,158].

Biodigestion may be divided into four main steps, according to the biochemical conversion of the organic compounds—hydrolysis, acidogenesis, acetogenesis, and methanogenesis—wherein at least five microbial groups participate in the process. Figure 2 depicts a simplified diagram of the anaerobic process, in which hydrolytic and fermentative bacteria initially convert complex substrates, i.e., polymers, such as carbohydrates and proteins, into short-chain compounds, such as volatile fatty acids (VFAs), alcohols, CO_2, and hydrogen (H_2). Acetogenic bacteria further convert VFAs (other than acetic acid) and alcohols into acetic acid, CO_2, and H_2, which correspond to the primary substrates utilized by methanogenic archaea (methanogens). Hydrogenotrophic methanogens grow autotrophically on CO_2 and H_2 to produce CH_4, while acetate is used as the carbon source by acetoclastic methanogens. Table 1 shows some relevant reactions carried out by the different microbial groups in the anaerobic conversion process.

Many factors directly affect the biodigestion process, which are strictly related to operating parameters that include pH, alkalinity, organic loading rate (OLR, i.e., the amount of substrate supplied to the microorganisms), nutrient supplementation, and temperature [159–161]. The presence of toxic compounds (e.g., phenolics naturally found in wastewaters or ammonia/sulfide released during substrate conversion) and the characteristics of the organic substrate, i.e., biodegradability and recalcitrance, also play important roles in the anaerobic process. Particularly for sugarcane vinasse, two primary factors must be considered: pH/alkalinity and temperature conditions. The high biodegradable organic fraction of sugarcane vinasse, especially in terms of carbohydrates (Table S1) and glycerol, promptly stimulates the activity of acidogenic bacteria, which present high growth rates (with a minimum doubling time of about 30 min [151]). Consequently, the rapid acidification of the wastewater may seriously affect the global anaerobic process [162], because methanogens (with a minimum doubling time of 2–3 days for acetoclastic methanogens [151]) are susceptible to the accumulation of VFAs [159]. Providing adequate alkalinity levels is imperative to prevent unbalancing conditions between acidogenic bacteria and methanogens.

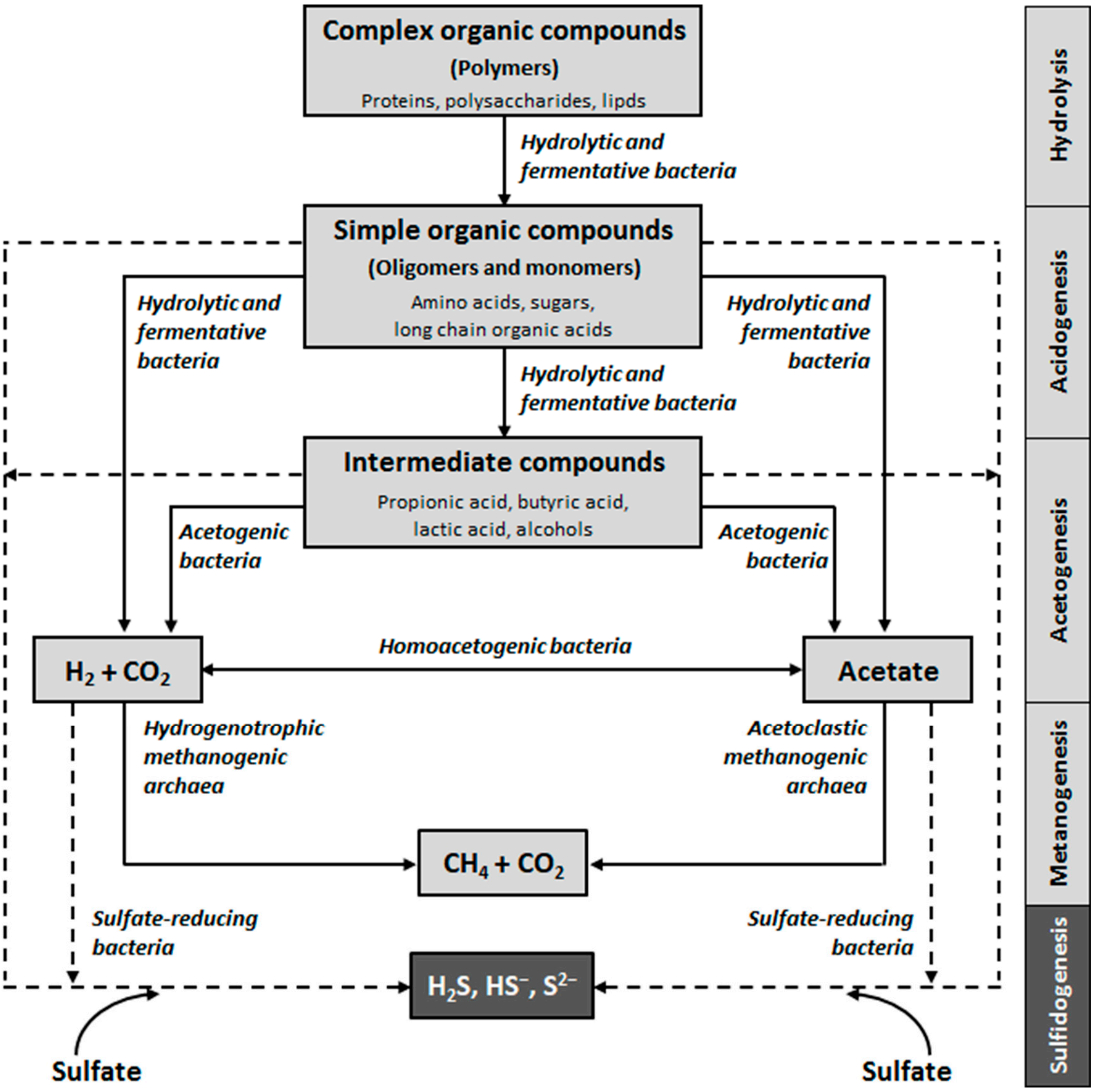

Figure 2. Simplified diagram of the anaerobic bioconversion of organic matter into methane, including the main involved microbial populations. Bold lines indicate the process without the interference of sulfate reduction; dashed lines indicate possible biochemical pathways associated with the reduction of sulfate into sulfide. Note: The diagram is based on McCarty and Smith [150] and Chenicharo [160].

Regarding the temperature, the application of thermophilic conditions (50–55 °C) may be considered the most suitable option for biodigestion systems used for sugarcane vinasse, based on the following relevant advantages: [i] vinasse is collected from distillation columns at temperatures as high as 90 °C [3,163], wherein cooling systems may be excluded as vinasse naturally reaches the required temperature conditions in intermediate transportation steps; and [ii] thermophilic systems support the application of higher OLR and present equivalent efficiencies compared to mesophilic systems, which leads to the installation of more compact reactors [23,47].

Finally, the high concentrations of sulfate observed in sugarcane vinasse are also a matter of concern when using biodigestion [79,81]. Applying sulfate-rich wastewaters to anaerobic systems stimulates the sulfidogenic pathway (Figure 2), in which sulfate is reduced primarily into sulfide (H_2S) by a specific group of microorganisms, namely, the sulfate-reducing bacteria (SRB) [164,165]. SRB are characterized as an extremely versatile microbial group insomuch as they outcompete methanogens when using common substrates, such as acetate and H_2 [159]. This characteristic limits the extraction of energy from organic matter by CH_4 production. Moreover, the high toxicity of H_2S may also affect the methanogenic activity in anaerobic systems, either by permeating microbial cells as the non-ionized form (H_2S) or by the precipitation of essential metals as the ionized forms (HS^- and S^{2-}) [159]. Kiyuna et al. [87] studied the influence of the COD/sulfate ratio on the biodigestion of sugarcane vinasse, and indicated that methane production is negatively affected by high sulfate concentrations. Conversely, these authors reported that the COD removal was not affected by sulfidogenesis, and also highlighted that the conversion of acetate improved when higher sulfate concentrations were applied. These observations also result from the versatility of SRB, which may act as acetogenic bacteria by partially oxidizing VFAs to acetate (Table 1) [154,166], and thus kinetically favor the activity of methanogens.

Table 1. Biochemical reactions performed by different microbial populations in anaerobic environments and the respective Gibbs free energies.

Microbial Group	Reaction	$\Delta G^{\circ\prime}$ (kJ mol^{-1})	Equation
Fermentative bacteria	$\text{Glucose} + 2H_2O \rightarrow 2\text{Acetate} + 4H_2 + 2CO_2$	−206.0	(1) [1]
	$\text{Glucose} \rightarrow \text{Butyrate} + 2H_2 + 2CO_2$	−254.0	(2) [1]
	$\text{Glucose} + 2H_2 \rightarrow 2\text{Propionate} + 2H_2O$	−279.4	(3) [1]
	$\text{Glucose} \rightarrow 2\text{Lactate} + 2H^+$	−198.0	(4) [2]
	$\text{Glucose} \rightarrow 2\text{Ethanol} + 2CO_2$	−164.8	(5) [3]
	$\text{Glucose} + H_2O \rightarrow \text{Ethanol} + \text{Acetate} + 2H_2 + 2CO_2$	−205.2	(6) [3]
	$\text{Acetate} + 2\text{Lactate} \rightarrow H_2 + 1.5\text{Butyrate} + 2CO_2 + H_2O$	−156.6	(7) [4]
	$3\text{Lactate} \rightarrow 2\text{Propionate} + \text{Acetate} + CO_2 + H_2O$	−170.0	(8) [5]
Acetogenic bacteria	$\text{Propionate} + 2H_2O \rightarrow \text{Acetate} + 3H_2 + CO_2$	+76.2	(9) [3]
	$\text{Butyrate} + 2H_2O \rightarrow 2\text{Acetate} + 2H_2$	+48.4	(10) [3]
	$\text{Ethanol} + H_2O \rightarrow \text{Acetate} + 2H_2$	+9.6	(11) [3]
	$\text{Lactate} + 2H_2O \rightarrow \text{Acetate} + 3HCO_3^- + 2H_2$	−4.2	(12) [3]
	$\text{Glycerol} + 2H_2O \rightarrow \text{Acetate} + HCO_3^- + 2H^+ + 2H_2$	−73.2	(13) [6]
Homoacetogenic bacteria	$4H_2 + 2CO_2 \rightarrow \text{Acetate} + 2H_2O$	−104.0	(14) [3]
Acetoclastic methanogens	$\text{Acetate} \rightarrow CH_4 + H_2O$	−31.0	(15) [3]
Hydrogenotrophic methanogens	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−135.0	(16) [3]
Sulfate-reducing bacteria	$SO_4^{2-} + \text{Acetate} \rightarrow HS^- + 2HCO_3^-$	−47.3	(17) [7]
	$SO_4^{2-} + 2\text{Butyrate} \rightarrow HS^- + H^+ + 4\text{Acetate}$	−55.5	(18) [7]
	$SO_4^{2-} + 2\text{Ethanol} \rightarrow HS^- + 2H_2O + H^+ + 2\text{Acetate}$	−132.7	(19) [7]
	$3SO_4^{2-} + 4\text{Propionate} \rightarrow 3HS^- + 4HCO_3^- + H^+ + 4\text{Acetate}$	−150.6	(20) [7]
	$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$	−152.2	(21) [7]
	$SO_4^{2-} + 2\text{Lactate} \rightarrow HS^- + 2HCO_3^- + H^+ + 2\text{Acetate}$	−160.1	(22) [7]
	$3SO_4^{2-} + 2\text{Ethanol} \rightarrow 3HS^- + 4HCO_3^- + H^+ + 2H_2O$	−227.3	(23) [7]
	$\text{Glycerol} + 1.25SO_4^{2-} \rightarrow 0.5\text{Acetate} + 1.5H_2CO_3 + 0.5HCO_3^- + 1.25HS^- + 0.75OH^- + H_2O$	−424.5	(24) [8]

[1] Mosey [151]. [2] Anzola-Rojas et al. [167]. [3] Saady [168]. [4] Matsumoto and Nishimura [169]. [5] Seeliger et al. [170]. [6] Thauer et al. [171]. [7] Zhou and Xing [172]. [8] Bertolino et al. [173].

4.2. Biodigestion of Vinasse in Brazil: Background and Current State of the Research

The anaerobic processing of organic residues and wastewaters has two primary targets: the bioenergy recovery from the methane-rich biogas (biogas-CH_4) produced by methanogens and the minimization of the polluting load of residues. In this context, various reactor configurations and operating conditions were proposed for the vinasses of various raw materials [23] in an effort to optimize the energy extraction mainly from single-phase systems, i.e., in which all the steps of the anaerobic process (Table 1) are carried out in a single reactor. However, studies may still be considered incipient when using biodigestion in the processing of sugarcane vinasse in Brazil, especially in terms of full-scale experiences. Initial studies were reported in the early 1980–1990s [34,37,174,175], and then a gap of approximately 20–25 years was observed until complementary relevant studies were made available [33,35,36,38,39,59,60,176–178]. The relatively recent growing interest in vinasse biodigestion was motivated by the search for alternative bioenergy sources and the need for reducing negative environmental impacts from fertirrigation. The ever-increasing concern for the environment has begun to critically assess any and all aspects of biofuel production chains, and the environmental burden of fertirrigation has gained considerable prominence in this context.

The low adherence of distilleries to the biodigestion of vinasse directly resulted from unsuccessful experiences after implementing full-scale biodigestion plants in biorefineries in the past, such as the METHAX Dedini® system, which resulted from a consortium (Codistil-Dedini/PEM/Paques) in the early 1980s [179]. The METHAX Dedini® system included a sludge-blanket reactor coupled to a biogas purification plant for producing biomethane (bioCH_4) to be used primarily as an automotive fuel. Despite the robustness of this system, its relative black box character, as well as the lack of fundamental knowledge in terms of plant operation and specificities of vinasse bioconversion seriously affected the acceptance of this process as a reliable technological approach for managing vinasse in Brazilian sugarcane biorefineries. The susceptibility to fast acidification and the requirements for long HRT [3,48,162] represent some of the specific points to be carefully considered. Moreover, prohibiting the sale of electricity imposed on biorefineries until the mid-1990s and the proven techno-economic (but not environmental) feasibility of fertirrigation were also preponderant factors that discouraged studies on the biodigestion and the energy exploitation of biogas from vinasse in Brazil [3].

The characteristics of some highly relevant (according to the suitability to industrial scale application) studies on sugarcane vinasse biodigestion from the Brazilian sugar and ethanol industry are listed in Table S3, which especially consider the achievements of each proposed system. Studies based on the processing of vinasses resulting only from sugarcane molasses, e.g., Acharya et al. [180], España-Gamboa et al. [181], Fernández et al. [182] and Kumar et al. [183], were not considered for comparison purposes, because the direct use of molasses for ethanol production is not carried out in Brazil. Blending molasses with juice or using pure sugarcane juice in fermentation (i.e., annexed and autonomous distilleries, respectively) generates considerably less concentrated vinasses than using molasses as the only substrate [17,23,88], and requires different operating strategies for optimizing the biodigestion systems. One of the main differences refers to the HRT, because lower values can be more easily implemented in the case of less concentrated vinasses. Initial studies on the biodigestion of sugarcane vinasse in Brazil showed promising results with the application of pilot systems, as presented in Craveiro et al. [34], Souza et al. [37] and Costa et al. [174] (Table S3). Despite the temporal gap in terms of relevant studies, recent reports [33,35,36,38–40,176–178] (most listed in Table S3) have shown significant improvements regarding the biodigestion of sugarcane vinasse, i.e., obtaining satisfactory COD removals (>70%) by associating high OLR (25–30 kg COD m^{-3} day^{-1}) with low HRT (<24 h). Nevertheless, these results were mostly obtained in bench-scale systems. These promising results may be attributed to two primary factors, namely, using thermophilic temperature conditions and phase separation for the systems.

Regarding future steps, the scale-up of new reactor configurations, such as the anaerobic structured-bed reactor (ASTBR) [28,36] and the optimization of using alkalizing compounds, is characterized as an essential step for encouraging the implementation of full-scale biodigestion plants in sugarcane biorefineries. Using fixed-film systems with high bed porosity (such as the ASTBR) tends to be more suitable than sludge-blanket reactors in the case of sugarcane vinasse, because specific compositional aspects (e.g., the relatively high contents of solids and salts) hinder an efficient granulation [155]. In terms of the system scale-up, the literature currently indicates the long-term operation (>20 years) of only one full-scale reactor (5200 m^3) in the Brazilian sugar and ethanol industry [179,184], which is used for processing approximately 25% of the vinasse (37 m^3 h^{-1}) generated in a large scale annexed biorefinery (milling capacity of 10 million tons of sugarcane -MTC- per harvest). Although the design of this reactor was based on the system proposed by Souza et al. [37], which successfully reported the operation of a pilot-scale (75 m^3) thermophilic reactor for over 250 days at high OLR (26.5 kg COD m^{-3} day^{-1}; Table S3), the implemented full-scale system operates far below the potential (OLR = 5 kg COD m^{-3} day^{-1}) [179]. Recent interest on the application of vinasse biodigestion (frequently associated with the co-digestion of filter cake, which depends on low rate reactors) is on course in Brazil; however, the results of the experiences will be observed in the coming years. In any case, the scale-up of efficient anaerobic systems is imperative for identifying real-life operating drawbacks, as well as for precisely assessing the energetic potential of the biogas from sugarcane vinasse. In fact, all the data concerning the assessment of the energy potential of vinasse (see Section 4.3) still derive from estimates, meaning that the potentials and limitations of biogas as a source of bioenergy in biorefineries will only be fully understood by pilot- and full-scale experiences [48].

Finally, the alkalinization of full-scale systems directly affects the economic feasibility of the biodigestion plants, as was recently described elsewhere [50,163]. Sodium bicarbonate ($NaHCO_3$) is often used as the alkalizing agent in studies reporting the biodigestion of sugarcane vinasse [35,36,59,60]. However, even low doses of $NaHCO_3$ (0.28–0.36 g $NaHCO_3$ g^{-1}COD) [163] lead to highly unfavorable economic scenarios. Biogas production costs in biodigestion systems using $NaHCO_3$ as the alkalizing compound may be up to 70-fold higher compared to using sodium hydroxide (NaOH) [50]. In this context, although alternative alkalizing approaches have been proposed, such as using urea [65,162,185], using NaOH may be the most feasible option [163]. Low doses of NaOH (4 g NaOH kg^{-1} COD) may provide adequate conditions for the bioconversion of the organic matter in high-rate reactors, as referenced by the results obtained by Souza et al. [37] (Table S3). Using effluent recirculation for alkalinity recycling is also characterized as a feasible option, as attested by a few pilot- and full-scale experiences [37,133] and economic assessments [50].

4.3. Predicting the Energetic Potential of Sugarcane Vinasse

The lack of full-scale experiences regarding the biodigeston of sugarcane vinasse in Brazil limits the assessment of its energetic potential in terms of practical results, as aforementioned. When considering the only case reported in the literature for the Brazilian industry [179], biogas is used for yeast drying in a spray dryer (8 tons of dried yeasts per day). However, approaches based on theoretical calculations and scenario prediction have historically presented relevant results for assessing the energy recovery capacity of vinasse-derived biogas in Brazilian biorefineries [46,48,50,61–66,184,186–191], and have even surpassed the number of published experimental approaches. The use of scenarization-based studies both complements the lack of experimental/field data and encourages the implementation of full-scale plants to enhance the energy balance of distilleries. Table S4 shows data on the estimation of the energetic potential of sugarcane vinasse from different studies, with a focus on the generation of electricity by applying biogas-CH_4 in different prime movers.

The energetic potential estimated for sugarcane vinasses from different studies ranged from 0.0001 to 2.61 MJ L^{-1} of ethanol (Table S4). The huge data variability directly results from the different assumptions considered in each study, primarily the specific vinasse production (8.14–15 L vinasse L^{-1} of ethanol), the type (internal combustion engines, gas and steam turbines) and conversion efficiency (23–43%; which depends on the plant size, the implementation of heat recovery, and also on the type) of the prime movers (Table S4). The type of feedstock used in ethanol production (juice or blends of juice and molasses), the organic content of sugarcane vinasse (COD = 21–45.8 g L^{-1}, in addition to one case considering the co-processing of vinasse and filter cake [192]), and the lower heating value (LHV) of the biogas (or methane; at 20.02–39.62 MJ Nm^{-3}) (Table S4) also influenced the obtained values. Nevertheless, the results indicate that the energy from biogas can offset the consumption of fossil fuels in distilleries, which corresponds to an average of 0.47 ± 0.20 MJ L^{-1} of ethanol, based on data available elsewhere [193–200]. A global analysis of the energetic potential data suggests that the values obtained from simple energy balances (>1.0 MJ L^{-1} of ethanol [48,61,62,65,184]) may be overestimated, because such studies do not usually include detailed characteristics of biorefineries and prime movers (Table S4).

Regarding the application of the energy recovered from biogas–CH_4, the studies propose numerous options. Fuess et al. [50] and Moraes et al. [63] indicated that the electricity produced from biogas could supply populations of approximately 130–140 thousand inhabitants during the sugarcane harvesting period, for biorefineries processing 4 and 2 MTC, respectively, per harvest. The operation of biodigestion-power plants during the inter-harvest could further supply 60 thousand inhabitants [50]. The aforementioned studies also highlighted that the energy from biogas could significantly improve the generation of surplus electricity in biorefineries, because more than 50% of the electricity exported to the grid could be obtained from biodigestion-power plants. Salomon et al. [64] reported that the generation of electricity from biogas in engines could lead to more favorable economic scenarios compared to the application of microturbines, although the most profitable option was characterized by co-firing biogas and bagasse in boilers. These authors also highlighted the high profitability of using biogas as fuel in spray dryers for yeast drying (further description on economic aspects is provided in Section 4.3.1). In global terms, i.e., considering the conversion of the total volume of sugarcane vinasse generated in Brazil into biogas–CH_4, the installed capacity reached values in the range of 819–1603 MW [46,49,50,63,184], depending on the assumptions used in each study. Although directing the entire amount of vinasse to biodigestion may not represent a factual scenario (because implementing biodigestion plants may not be economically attractive in small-scale sugarcane processing plants), these values correspond to 7.0–13.7% of the installed capacity of electricity generation from bagasse (11,712 MW [201]). Even more favorable scenarios could be obtained using the consumption of fossil sources as a reference, as up to 49.2% and 50.0% of the electric power of fuel oil and coal (3256 and 3203 MW [201]) could be respectively replaced by the energy from biogas.

Improvements in terms of energy extraction by applying two-phase biodigestion to the processing of sugarcane vinasse may also be highlighted from data shown in Table S4, because the electric power of biogas–CH_4 (7.0–10.8 MW [50]) reached levels between 10–115% higher compared to single-phase systems (5.02–6.37 MW [63,64]). The operation of biodigestion-power plants during the inter-harvesting period (off season) also greatly improved electricity production from vinasse, and led to electric power values of 12.3 MW and 15.5–16.3 MW for single- and two-phase biodigestion schemes, respectively. The use of gas turbines coupled with steam turbines (combined cycle) was considered in this case [50]. The unavailability of sugarcane vinasse during the off season (once ethanol production is interrupted) is one of the main bottlenecks hindering efficient high rate biogas production in vinasse-fed plants. Defining strategies to achieve year-round operation, as proposed elsewhere [50,52,53], represents one of the main topics to be addressed in new vinasse-based research within the context of biodigestion. In particular, Fuess et al. [50] also reported estimates concerning the recovery of thermal energy from biogas in engines and gas turbines with values of 6.8–8.3 MW and 3.4–4.2 MW for the harvesting and inter-harvesting periods, respectively. Although these values (8.17–9.94 kWh TC^{-1} [50]) are considerably lower than the consumption of steam in distilleries (300 kWh TC^{-1}), the association between electricity production and thermal energy recovery could lead to marked improvements in terms of biogas conversion, with values as high as 86.7% when using gas turbines as the prime movers [50]. In practical terms, the thermal energy recovered from biogas could be used for maintaining the temperature of the reactors [3], mainly in thermophilic systems.

4.3.1. Economics of Vinasse-Derived Bioenergy

Estimates on the energy production from sugarcane vinasse are frequently associated with economic assessments used to both measure profitability levels and identify key factors impacting process profitability (sensitivity analyses). Common tools used to assess the economic performance of anaerobic systems include the net present value (NPV), the internal rate of return (IRR), and the discounted payback period [49,50,63] when considering greenfield and brownfield scenarios. In the case of vinasse biodigestion, greenfield investments refer to the installation of an entirely new sugarcane biorefinery (i.e., including steps from sugarcane processing to biogas production and conversion), while brownfield ones address the implementation of anaerobic-power plants in operating biorefineries (i.e., the investments refer only to the infrastructure demanded by vinasse processing and biogas use). Consequently, potential revenues include sugar (in the case of annexed plants), ethanol, and biogas-derived energy in the first case, while only the biogas-derived energy is a marketable product in the latter. A direct comparison among different studies is hindered due to the use of different assumptions in each case, including technical (e.g., biorefinery size, quantitative and compositional characteristics of vinasse, type of anaerobic system, type and efficiency of the prime movers, etc.) and economic (e.g., minimum acceptable rate of return—MARR, project lifetime, tax rates, etc.) aspects. Nevertheless, results indicate that the production and use of biogas from vinasse can be economically feasible in both greenfield and brownfield approaches, provided that the following conditions are achieved: NPV > 0, IRR > MARR and payback period < project lifetime.

Considering brownfield scenarios, Salomon et al. [64] reported on the economic feasibility of using vinasse-derived biogas to produce electricity in reciprocating combustion engines or in cogeneration systems (coupled with the commercialization of surplus bagasse in the latter case), as well as to generate heat directed to yeast drying (in all cases: IRR > MARR = 15%). Electricity generation in micro gas turbines was demonstrated to be unfeasible in this case (IRR = 7.06%). In another brownfield approach, Fuess and Zaiat [49] also observed the economic feasibility (IRR > 12% vs. MARR = 8.76%) of three different biogas uses in annexed biorefineries, namely, the enhanced electricity generation in combined cycle power plants, the cogeneration of electricity in boilers, and the production of $bioCH_4$ for grid injection. The authors demonstrated that achieving process profitability in autonomous plants (IRR < 12%) would directly depend on the type of biodigestion plant, i.e., single- or two-phase. The use of phase separation would negatively impact process profitability due to the higher investment costs required to build facilities processing higher vinasse flow rates (compared to the annexed plants). The profitability of electricity production from biogas in engines was associated with a wide range of electricity prices (from USD 59.83 to USD 76.93) by Nogueira et al. [189], with IRR values directly dependent on the operating period considered for the power plant.

With respect to greenfield investments, the results usually show profitable scenarios regardless of the biogas use, because of the high market values of ethanol and sugar (which represent the main biorefinery products in this type of approach). Moraes et al. [63] observed that the use of biogas for electricity production (using either boilers or internal combustion engines) or for diesel replacement could provide some extra revenue in autonomous ethanol plants. However, IRR values (13–14%) were relatively low compared to the IRR (12%). In this case, annexed biorefineries were demonstrated to be almost economically unfeasible due to higher investment costs. In contrast, Fuess et al. [50] showed that electricity production from biogas in annexed plants could be highly profitable (IRR of ca. 20% vs. MARR = 12%) by using different prime movers (internal combustion engines, gas turbines, or the combined cycle). The operation of the biorefinery as an autonomous plant processing energy cane during the inter-harvest directly increased revenues with electricity. The authors also demonstrated the significant negative impacts of dosing alkalinizing agents ($NaHCO_3$) on the economic performance of the entire biorefinery. Implementing a flex ethanol facility processing sugarcane (harvest) and maize (inter-harvest) also was demonstrated to be an attractive strategy to improve the economic performance of sugarcane biorefineries [192]. While relatively low IRR values (ca. 15%) were estimated for electricity production, bioCH_4 production and use (grid injection or diesel replacement with IRR values of ca. 25% and 40%, respectively) would be the most profitable option in this case (considering MARR = 12%). Additional details on the production of bioCH_4 from vinasse can be found in Section 4.6.

The complementary use of sensitivity analyses indicates the main factors impacting the profitability of bioenergy production from vinasse, which is highly useful to identify investment opportunities and provide guidelines for policy makers within the biofuel industry context. Overall, investment costs and the product (electricity or bioCH_4) prices impact the profitability to a higher extent than other factors, such as the operating costs [49,66,189]. In practical aspects, these results indicate that both the agents controlling tax rates (governments) and the market demands directly impact the economics of biogas production and use.

4.4. Phase Separation: Enhancing Resource Recovery from Vinasse

The use of biodigestion with phase separation aims to optimally exploit the potentials of different microbial groups acting in the bioconversion of organic matter, primarily hydrolytic and fermentative bacteria in the acidogenic phase (Equations (1)–(8); Table 1) and methanogenic archaea (Equations (15) and (16); Table 1) in the subsequent methanogenic phase. Despite breaking some syntrophic relationships among the microbial populations (Figure 2) [202], phase separation brings numerous advantages, including [i] optimization of potentially limiting anaerobic conversion steps, such as the hydrolysis of complex materials in the acidogenic phase and the consumption of acids by acetogens and methanogens; [ii] enhancement of operating stability by preventing the exposure of methanogens to the accumulation of VFAs, which also dramatically reduces the requirements of alkalizing compounds; [iii] enhancement of both the substrate biodegradability and energy extraction from organic residues; and [iv] reduction in the start-up period coupled with increasing processing capacities, i.e., the application of high OLR for methanogenic reactors [35,202–207]. In particular, these characteristics suggest that drawbacks resulting from the susceptibility of vinasse to rapid acidification [162] could be successfully offset in two-phase biodigestion schemes. Moreover, phase separation may also characterize a strategy to prevent the accumulation of VFAs in thermophilic systems as the activity of acidogenic bacteria is enhanced at higher temperatures [206].

The reference literature is still poor in terms of using two-phase biodigestion of sugarcane vinasses from Brazilian distilleries, with a few recent studies available [35,36,177], all detailed in Table S3, in addition to Ramos et al. [39]. However, the results reported in these cases, particularly by Ferraz Jr. et al. [35] and Fuess et al. [36], indicated the technical feasibility of vinasse bioconversion in two-phase systems, as both the organic matter removal and energy extraction through methane could be markedly enhanced when applying high OLR and relatively low HRT (Table S3). Complementary studies based on estimates and simulations of biodigestion systems applied to vinasse further confirmed the economic and environmental feasibility of scaling-up two-phase schemes in sugarcane biorefineries [49,50,163]. Fuess et al. [163] pointed out that the costs of producing biogas and electricity obtained in two-phase biodigestion systems can reach equivalent or lower values compared to single-phase layouts. In this case, the higher capital and operating costs intrinsic to two-phase schemes were demonstrated to be offset by the savings resulting from much lower consumption of chemicals. Fuess et al. [50] later associated lower environmental impacts with two-phase biodigestion systems applied to sugarcane vinasse, provided that an optimized use of chemicals in the reactors is observed.

The benefits of phase separation in anaerobic processes extend far beyond enhanced bioenergy recovery through CH_4 production, as the acidogenic step may be optimized for the recovery of a series of bioproducts, such as biohydrogen (bioH_2), organic metabolites (in particular, short-chain organic acids and solvents) and biopolymers. Figure 3 depicts different routes for processing vinasse through two-phase biodigestion in sugarcane biorefineries, in which the methanogenic step may be potentially coupled with different stages of the global process. In terms of practical results, the production of bioH_2 from sugarcane vinasse has been extensively studied in Brazilian research groups in recent years [44,77,79,208–222], while studies on the additional pathways considered in Figure 3, i.e., enhanced production of metabolites and biopolymers, are still relatively scarce or non-existent.

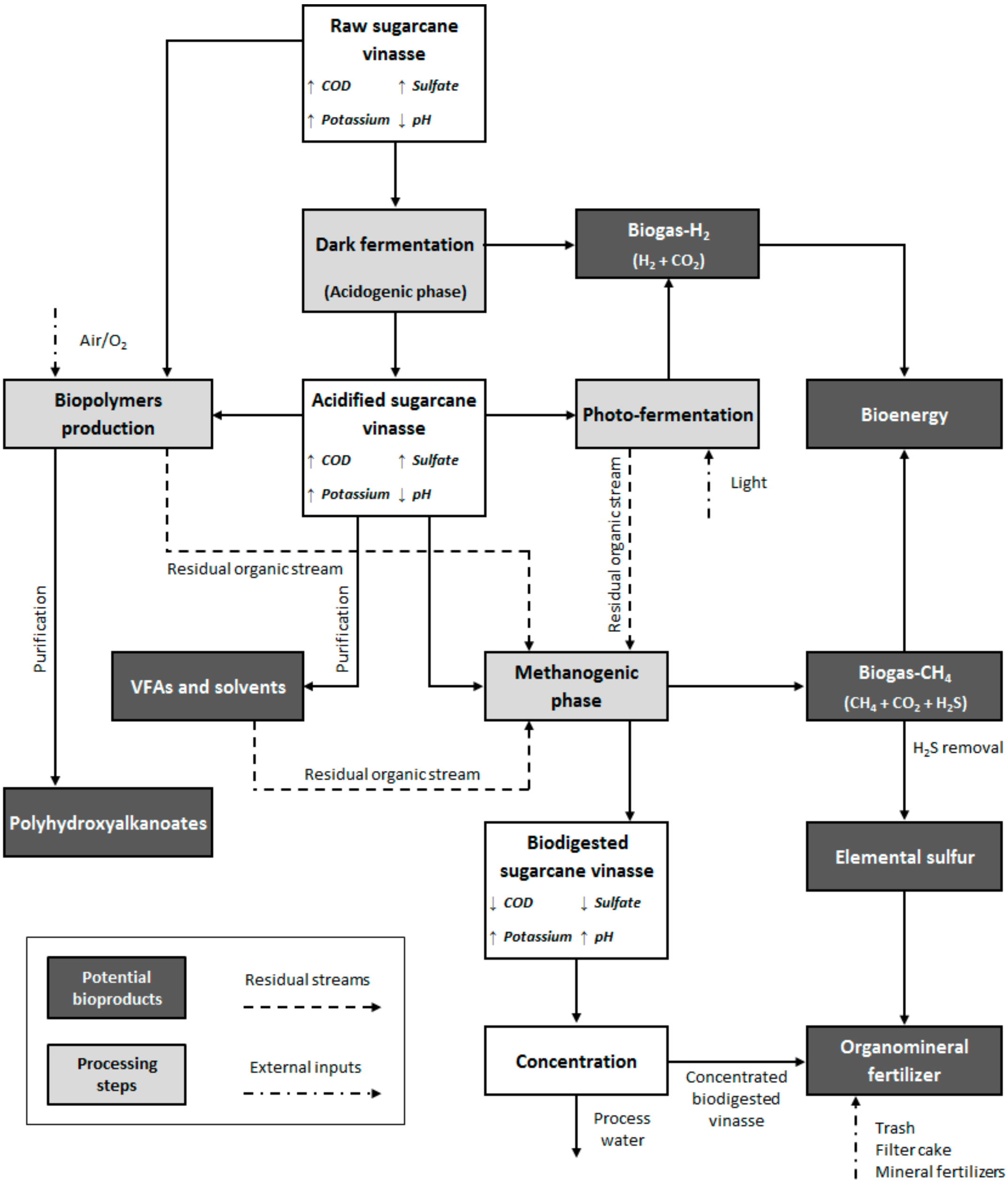

Figure 3. Potential technological routes for the exploitation of sugarcane vinasse using biodigestion with phase separation. (↑) means "high values", while (↓) means "low values".

Efforts in using H_2 as a bioenergy carrier consider two primary factors: its excellent fuel properties, such as high energy density (120 MJ kg^{-1} [223]), rapid burning speed, and low minimum ignition point [224]; and the generation of water as the only "residue" from burning [203,225]. Moreover, the biological production of H_2 could offset the energetic and environmental drawbacks inherent to conventional electro- and thermochemical production processes, which are characterized by the intense use of fossil resources [67,226]. Investigations on bioH_2 production, which comprises the gas stream evolved from the acidogenic phase together with CO_2 (Figure 3), i.e., biogas–H_2 (Equations (1), (2), (6), and (7); Table 1), may still be considered fundamental in an attempt to identify adequate conditions to achieve continuous and stable long-term production profiles. Table S5 shows characteristics of the studies on bioH_2 production from sugarcane vinasse in Brazil, while also considering the main aspects of each case. BioH_2 production may be carried out according to different pathways, including photosynthetic, photo-fermentative and exclusively fermentative (dark fermentation) processes [41,225]. Dark fermentation corresponds to the acidogenic phase of the biodigestion process (Equations (1)–(8), Table 1; Figure 2), in which adequate conditions of substrate availability and HRT are provided to stimulate the activity of fermentative bacteria rather than methanogens. In turn, bioH_2 production from photosynthetic processes may be carried out either directly (i.e., using the metabolism of algae, protists, and photosynthetic bacteria to convert water into chemical energy) or indirectly (i.e., growing purple non-sulfur bacteria on VFAs resulting from acidogenesis; Equations (25) and (26)) [41,67,213,225,227].

$$\text{2Acetate} + 4H_2O + \text{Light} \rightarrow 8H_2 + 4CO_2 \tag{25}$$

$$\text{Butyrate} + 3H_2O + \text{Light} \rightarrow 10H_2 + 4CO_2 \tag{26}$$

Studies on the application of dark fermentation to sugarcane vinasse are more numerous than light-based processes (Table S5), and present a few common characteristics, such as the preference for thermophilic temperature conditions (55 °C) and biofilm systems (fluidized- and fixed-film reactors). High temperatures thermodynamically favor the production of bioH_2 [209,224], and the reduced solubility of gases in such conditions also enhances the release of bioH_2 to the gas phase. In practical aspects, using fixed-bed reactors in the acidogenic phase for processing sugarcane vinasse has led to important achievements, such as defining optimized OLR [77,209] and suitable support material [211], as well as implementing operating strategies to maintain long-term bioH_2 production rates [44] (Table S5). The strict dependence of fermentation on the pH has also been demonstrated with the favoring of lactate accumulation ($pH < 5.0$), the production of bioH_2 and butyrate with lactate consumption ($pH = 5.0$–5.5), or the production of the latter coupled with sulfate reduction ($pH > 6.0$) [77,79]. The fermentative microbial groups respond differently to different pH levels, which directly impact the distribution of soluble metabolites. Moderate-to-high acidic conditions ($pH < 5.0$) are suitable for the growth of a limited number of microbial groups (among them, lactate-producing bacteria [77]) and are, therefore, highly unsuitable for most microbes (such as those consuming lactate to produce butyrate and other organic acids). Meanwhile, a more diversified metabolite distribution can be expected at higher pH values (>6.0), which may include the participation of propionate-forming bacteria [43] in association with butyrate- and acetate-producing groups and non-fermentative bacteria, e.g., sulfate-reducing groups. Overall, suitable pH conditions are maintained through dosing chemicals (neutralizing and/or alkalinizing agents) in reactors, which is similar to conventional methanogenic reactors. In the particular case of sugarcane vinasse, dosing NaOH is the main pH control approach used [44,77,79,209–211,215].

Fluidized-bed systems also show a great potential for the production of bioH_2 from sugarcane vinasse, and usually present the highest volumetric production rates (Table S5), which is most likely due to the enhanced gas–liquid separation triggered by the high recirculation rates. However, some limitations may affect the scale-up of these systems, which primarily include the need to dilute vinasse to prevent negative effects from organic overloads and the high energy consumption associated with the fluidization of the bed (Table S5). The characterization of microbial communities involved in bioH$_2$ production from sugarcane vinasse was also investigated in recent studies [209–213,219–222,228] in an effort to associate optimal operating conditions with specific microbial groups, such as those belonging to the *Thermoanaerobacterium* genus. Despite the marked progress, some aspects still require careful attention for an efficient bioH$_2$ production from sugarcane vinasse, and these include [i] the definition of suitable reactor configurations, especially considering the improvement of fixed-bed reactors through the application of ASTBR systems; [ii] the adequate control of biomass concentrations within the reactors; and [iii] the adequate control of the pH of the systems coupled with the rational use of chemicals. In turn, light-based processes have further limitations, such as large areas requirements (primarily to offset the negative effects of the high color of vinasse) and external inputs of luminous energy [41,67,205]. Nevertheless, practical results indicate that the application of photo-fermentative processes to acidified effluents may enhance bioH$_2$ yields by at least 50% of the values usually reported for dark fermentation systems [41], removing organic compounds from the wastewater.

Although the aforementioned aspects still hinder an efficient recovery and exploitation of bioH$_2$ at larger scales, Fuess et al. [50] simulated different alternatives for the use of bioH$_2$ in sugarcane distilleries in an attempt to enhance the bioenergy extraction from vinasse, which included biogas–H$_2$ (i.e., bioH$_2$ + CO$_2$) purification and sale of the bioH$_2$ as a value-added product and biogas–H$_2$ blending with biogas–CH$_4$ for biohythane production and biogas–H$_2$ purification, followed by the injection of bioH$_2$ into the methanogenic reactor for enhanced biogas–CH$_4$ production. The production of biohythane aims to improve the fuel properties of biogas–CH$_4$ [203,229,230]. The term biohythane derives from the term hythane®, which is characterized by adding hydrogen (typically 10–25%, v/v) to methane-based fuels to improve specific properties of the gas, such as the flammability limit, flame speed, and temperature ignition [203,230–232]. In turn, the injection of bioH$_2$ into the methanogenic reactor aims to upgrade the biogas–CH$_4$ (i.e., increase the CH$_4$ content) by stimulating the activity of hydrogenotrophic archaea (Equation (16), as seen in Table 1 [233–235]. The production of biohythane was characterized as the best option, and led to energetic gains compared to the use of biogas–CH$_4$ without requiring the previous purification of the biogas–H$_2$ stream [50]. Injecting purified biogas-H$_2$ into the methanogenic reactors could also lead to similar energetic gains compared to using biohythane. However, the purification step would require additional capital and operating costs [50]. Using phase separation exclusively to increase the vinasse biodegradability in the subsequent methanogenic step also proved to be an attractive alternative to such an extent that the limitations in terms of bioH$_2$ recovery did not hinder the operation of an acidogenic reactor in full-scale biodigestion plants [50,163]. Figure 4 depicts the potential technological approaches to enhance the bioenergy recovery from bioH$_2$ in biorefineries, which may also include using fuel cells for the production of electricity and thermal energy.

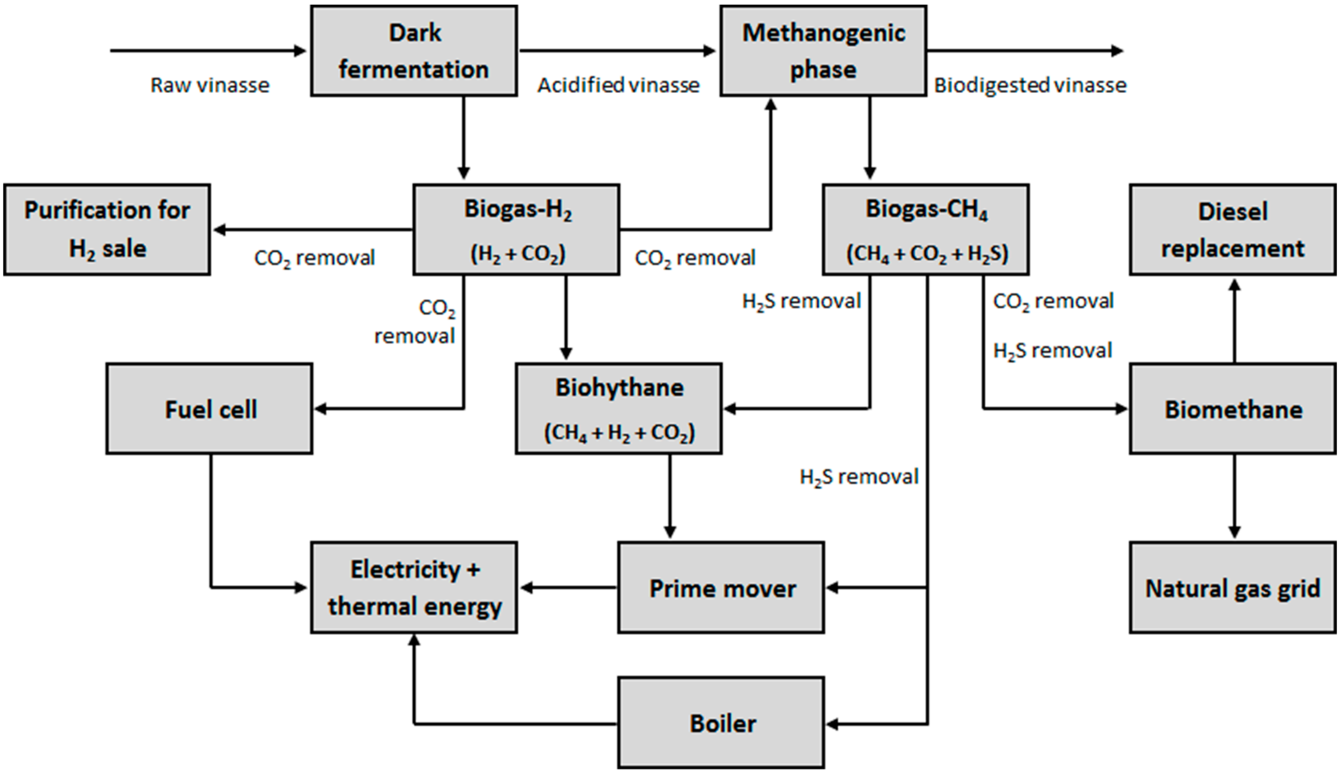

Figure 4. Potential applications for the biogas streams from biodigestion with phase separation.

4.5. Alternative Pathways for Bioresource Recovery from Vinasse via Acidogenesis

The optimization of acidogenic systems for recovering bioproducts from the liquid phase, i.e., organic acids and solvents (Figure 3), usually impacts the recovery of bioH_2, based on the application of specific operating parameters to select target microbial populations and further enhance the production of soluble metabolites [43,236–238]. In other words, enhanced production of bioH_2 may not always be directly associated with the enhanced production of a given soluble metabolite. Solventogenic pathways should be observed at low pH values (4.0–4.5)—which are unfavorable for bioH_2-producing groups [77]—as a response of the microbial metabolism to the accumulation of VFAs [239], while the proportion of organic acids also depends directly on the type of substrate and operating conditions applied to the systems [43,205,214,240,241]. Organic acids may be directly used in food and chemical synthesis industries [205,240]. In turn, the production of solvents from residues, such as ethanol and butanol, directly increases the energy balance in biorefineries [241].

In particular, results from studies assessing the production of bioH_2 suggest the potential for recovering butyric acid from sugarcane vinasse (Table S5), although the optimization of the hydrogenogenic activity should focus on the acetic type fermentation (Equation (1), Table 1), in which the hydrogen yield is maximized. Butyric acid production from direct sugar fermentation (Equation (2), Table 1) and from the co-fermentation of acetate and lactate (Equation (7), Table 1) frequently prevails in the acidogenesis of vinasse [43,77,79,215,228,238]. Butyric acid concentrations in fluidized-bed reactors reached values often exceeding 2000 mg L^{-1} [214,217,220–222], while values within the range of 1400–6000 mg L^{-1} were observed in fixed-bed (both APBR and ASTBR) systems [44,77,79,209,210], depending on both the inoculation and operating conditions. Butyric acid may be used as raw material for the production of butanol, either via catalytic [242] or biochemical [243] routes. Butanol is a promising biofuel, and shows important advantages compared to ethanol. In addition to a 30% higher energy density, butanol may be blended with gasoline and diesel at higher proportions, due to the hydrophobicity of its hydrocarbon chain, and it also performs better for the cold start of engines [244–246]. The esterification of butyric acid in association with butanol produces butyl butyrate, an alternative jet fuel [205,242], while the production of esters for food flavoring is also a widespread application of butyric acid [205,247]. The production of butyric acid from biochemical routes is more advantageous than the conventional process (butyraldehyde oxidation), because the starting material for this process (propylene) is obtained from crude oil [205].

In contrast to bioH_2 production (characterized by numerous experiments dealing with continuous reactors; Table S5), the few recent studies focusing on the recovery of soluble metabolites from sugarcane vinasse are at an earlier stage of research, and are still based on experiments carried out in batch conditions. Moraes et al. [82] reported butyric and iso-butyric acid concentrations, as high as 10.60 and 10.34 g L^{-1}, respectively, when fermenting blends of sucrose and sugarcane vinasse using microbial inocula subjected to different pretreatment methods (thermal and acidic-thermal). In this case, vinasse was used primarily as a nutrient (rather than carbon) source. Sydney et al. [248] also exploited sugarcane vinasse as a nutrient source for the recovery of organic acids; however, the potential for the concomitant bioH_2 production was also considered. In this case, different supplementary carbon sources were assessed, including sucrose, sugarcane juice, and sugarcane molasses. High butyric acid concentrations were also reported by these authors, with values ranging from 6.3–8.0 g L^{-1}, 4.3–6.4 g L^{-1} and 4.8–7.0 g L^{-1}, respectively for juice, molasses, and sucrose as carbon sources. Fuess et al. [249] further observed butyric and iso-butyric acid concentrations within the ranges of 6.0–6.4 g L^{-1} and 5.8–6.4 g L^{-1}, respectively, using vinasse as the only carbon source and the same types of inoculum pretreatment reported elsewhere [82]. A simple energetic assessment carried out by Fuess et al. [249] demonstrated that enhanced butyric acid production could represent an energy recovery potential of 30.9–33.4 MW, which exceeded the numbers observed for highly efficient biohydrogen (<1.0 MW) and methane-producing (25.5 MW) biodigestion plants. Sánchez et al. [43] and Eng et al. [238] presented the systematic assessment of environmental factors impacting VFA production from sugarcane vinasse. Sánchez et al. [43] observed maximum acidification (regardless of the VFA types) at alkaline (pH = 8.8–10.0) and mesophilic (40 °C) conditions. Interestingly, acetate was the main metabolite found in the mesophilic range (30–40 °C), while butyric-type fermentation prevailed at higher temperature (55 °C). The authors suggested that investing in butyrate recovery from vinasse (in concentrations exceeding 3500–4000 mg L^{-1}) represents an economically more attractive option than maximizing the production of medium chain VFAs (e.g., caproic and valeric acids), which eventually demands exhaustive operating efforts.

Butyric acid, in association with other organic compounds, may also be used as a precursor in the production of polymers in biological processes (Figure 3). The production of biopolymers from wastewaters aims to reduce the production costs of bioplastics (up to 15-fold higher than conventional plastics [250]) in an effort to replace oil-derived polymers [251–254]. These compounds present mechanic and thermoplastic properties similar to conventional polymers, with an additional advantage characterized by their biodegradable character [68,205,255–258]. Polyhydroxyalkanoates (PHA) are the main group of biopolymers studied and are characterized as linear aliphatic polyesters accumulated as cytoplasmic inclusions in microbial cells subjected to conditions of essential nutrient shortages [251,252,256,259]. The production of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV), which are the most common PHA stored as internal carbon and energy reserve by microorganisms [251], strictly depends on the type of organic acids found in the substrate. Butyric and acetic acids are the precursors for the synthesis of PHB, while PHV is produced primarily from propionic and valeric acids [251,252].

Sugarcane vinasse could be used either *in natura* or acidified as a substrate for the production of biopolymers (Figure 3). However, a critical assessment of both types of vinasses suggests that better results could be obtained by using acidified streams. Butyric acid concentrations in raw vinasse are considerably lower than in acidified vinasse, with values being usually less than 1000 mg L^{-1} (Table S1). Similar patterns are observed for acetic and propionic acids (Table S1), which are also characterized as important precursors in the production of PHA. In fact, the production of biopolymers from carbohydrate-rich and/or VFA-poor effluents, such as raw vinasse, is less attractive than using acidified streams, because the supply of sugars to stressed cells favors the accumulation of glycogen as the energy storage instead of PHA [260]. In this context, the application of acidified sugarcane vinasse to the production of biopolymers could most likely result in high proportions of PHB, based on the suitability of vinasse for the production of butyric acid via acidogenic processes [82,248,249]. In turn, the production of PHV from vinasse could be coupled with acidogenic systems operated at high OLR, based on the enhanced production of propionic acid at such conditions, i.e., 4036–5026 mg L^{-1} (OLR > 360 kg COD m^{-3} day^{-1} [221]), 3280–4378 mg L^{-1} (OLR > 90 kg COD m^{-3} day^{-1} [222]), and >5000 mg L^{-1} (OLR = 360 kg COD m^{-3} day^{-1} [214]).

The production of PHA from sugarcane vinasse in the Brazilian sugar and ethanol industry is still poorly exploited, and only two experimental reports could be found up to the publication of this review. Using mixed microbial cultures and fermented vinasse, Oliveira et al. [261] observed maximum PHA storage yields (0.60 g COD-PHA g^{-1}COD) when applying an OLR of 4.5 kg COD m^{-3} d^{-1} in a sequencing batch reactor after selecting a PHA-accumulating consortium from activated sludge-derived inoculum. PHB (86–77%mol) was the prevailing PHA, as previously speculated for the case of fermented vinasse, while PHV (14–23%mol) accounted for the remaining fraction of the produced PHA. Silverio et al. [262] used concentrated vinasse (7°Brix) as the carbon and mineral source to produce PHB assessing two bacterial strains, namely, *Halomonas* sp. HG03 and *Ralstonia eutropha* L359PCJ in continuous stirred-reactor tanks (CSTR). In the case of *R. eutropha* L359PCJ, which grows on glycerol, vinasse, together with a concentrated glycerol solution, were used as the carbon sources, while in the case of *Halomonas* sp. HG03, vinasse was used mainly as a nutrient source, such that the carbon supply derived from a concentrated sucrose solution. A higher PHB proportion (66.3%) was found when cultivating *R. eutropha* L359PCJ, which was associated with a PHB yield of 0.32 g PHB g^{-1} of glycerol. *Halomonas* sp. HG03 yielded 0.16 g PHB g^{-1} of sucrose, with a PHB content of 42.2%.

The integration of PHB production into Brazilian sugarcane biorefineries was also proposed by Nonato et al. [255]; however, they considered a completely different approach based on using sugar as the carbon source. These authors proposed a PHB production model by using facilities, materials, and surplus energy from biorefineries, to significantly reduce PHB production costs. The application of 3.85 tons h^{-1} of sugar would lead to a PHB production of 1.28 tons h^{-1} (10,137.6 tons $year^{-1}$) and require 4147 kW of electricity and 50.56 tons h^{-1} of steam during the harvesting and inter-harvesting periods (330 days). The diversion of sugar for PHB production would not affect sugar stocks, nor would it significantly affect sugar prices, although approximately 17% of the total sugar produced in the mill would supply the PHB plant. Further assessments of biopolymer production in the context of sugarcane biorefineries were proposed by Amulya et al. [251] and Albuquerque et al. [260], while considering the application of acidified molasses and acidified sugarcane (molasses) vinasse as the substrates, respectively. Although these cases do not refer to the Brazilian sugar and ethanol industry, important results were obtained, especially considering the findings obtained by Amulya et al. [251]. These authors reported the successful production of PHA by *Bacillus tequilensis* with different proportions of PHB and PHV, which depended on the applied OLR (0.66–2.64 kg COD m^{-3} day^{-1}). PHA accumulation within the cells reached up to 40% of the dry cell weight, with an associated COD removal of up to 73%.

Despite the promising results from using sugarcane vinasse to produce organic metabolites and biopolymers, the recovery of target products via purification processes still negatively affects the economic feasibility of the global process that requires further massive research to optimize full-scale applications. The costs incurred with the recovery of organic acids from fermented substrates typically vary between 30–40% of the total production costs [263], while this value reaches approximately 30% in the recovery of biopolymers [250,253]. However, considering the context of biorefineries, the purification step of both organic acids and biopolymers could be economically and environmentally improved by using solvents, specifically fusel oil generated as byproducts from the fermentation step and recovered through distillation [255]. Bekatorou et al. [263] assessed the application of numerous solvents in the recovery of organic acids from fermented media, and reported recovery efficiencies for the iso-butyric and butyric acids of up to 78.2% and 75.0%, respectively, when using fusel oil. Regarding the environmental performance, the gains could result from the replacement of chlorine-based compounds, which are usually used in the purification of polymers [255].

Apart from focusing on the production and recovery of bioH_2 and organic value-added products, the potential to stimulate the establishment of SRB populations in vinasse-fed acidogenic reactors has recently (re)gained attention as an efficient approach to boost methane production in two-phase biodigestion. Sulfate constitutes one of the main compounds with the potential to negatively impact methanogenesis, as detailed in Section 4.1. Some characteristics of mesophilic (35 °C) vinasse-fed acidogenic-sulfidogenic reactors were first addressed in the early 1990s [264,265], with preliminary results (at relatively high HRT, i.e., 28.8 and 22 h) indicating the superior performance (enhanced sulfate removal coupled with acetate accumulation) of fixed-bed reactors compared to suspended cell growth ones (CSTR). A sharp decrease in the sulfidogenic activity at pH values below 6.2 was also observed in these studies. Operating conditions were not restricted enough to prevent the occurrence of methanogenic activity occurring mainly in the fixed-film reactor [264]. This indicates the importance of selecting adequate inocula to simultaneously reach three metabolic demands, which include [i] the removal of sulfate, [ii] the fermentation of easily degradable substrates, such as glycerol and carbohydrates (with both processes ideally stimulating the acetogenic activity), and [iii] the inhibition of methanogens.

After a gap of ca. 30 years, this topic was re-introduced after the observation of sulfidogenesis during the thermophilic fermentation of sugarcane vinasse when dosing $NaHCO_3$ to ASTBR systems [77,79]. A sulfate removal efficiency exceeding 80% at harsh operating conditions (HRT = 6 h and OLR = 100 kg COD m^{-3} day^{-1}) was observed, with results indicating the need to maintain adequate pH values (>6.0) as the only environmental requirement of sulfate-reducing groups. Meanwhile, the harsh operating conditions usually applied in vinasse fermentation (Table S5) directly inhibit methanogens. Within this context, the production of fermentative inocula from the natural fermentation of vinasse, as consistently carried out elsewhere [44,77,79,209–211,215], may be the key aspect to achieve such a "metabolic success", because specific SRB (genera *Desulfotomaculum* and *Desulfohalotomaculum* [238,266]) have the ability to sporulate and then resist high VFA accumulation, which is in contrast to methanogens. The definition of optimal operating conditions in both continuous sulfidogenic-fermentative and methanogenic reactors is still required to better understand the effective energy gains in the biomethanation of sulfate-free (and eventually acetate-rich) fermented vinasses. Nevertheless, batch experiments showed the potential to maintain methane proportions as high as 80–90% in biogas (compared to less than 70% in the processing of raw, and therefore sulfate-rich, vinasse), which led to a 14% increase in the lower heating value (LHV) of the biogas–CH_4 [81]. From an energy exploitation perspective, this is more representative than investing on bioH_2 production, whose contribution to the global energy potential of biodigestion tends to be lower than 5% [35,36].

4.6. Additional Opportunities for Sugarcane Vinasse Exploitation via Biodigestion

Using biodigestion to process sugarcane vinasse offers additional opportunities for recovering bioresources in biorefineries, regardless of applying two-phase schemes. Regarding the direct recovery of bioenergy through biogas, in addition to electricity and thermal energy production, the biogas–CH_4 stream evolved from methanogenic systems can replace diesel in engines as a vehicular fuel and can be injected into the natural gas grid (Figure 4) [3,46,49,63,192,267–270]. "Raw" biogas–CH_4, i.e., still containing CO_2 but free of H_2S, could be directly used to replace diesel, but only partially. The lower calorific power of CO_2-containing biogas (which results from the non-fuel character of CO_2) demands the use of diesel as a supplementary fuel source, and the amount of diesel required will depend on the CH_4 content in biogas [63]. Moraes et al. [63] estimated that the use of biogas-CH_4 containing 60% CH_4 could replace only 40% of the diesel used in the fleet of sugarcane biorefineries processing 2 MTC per harvest. Hence, a more efficient use (on both energetic and environmental terms) of biogas–CH_4 for such purposes, i.e., diesel and natural gas replacement, depends on a purification step. In practical terms, the upgrade of biogas–CH_4 to bioCH_4 is required to obtain a final CH_4 content of at least 90% and trace levels of H_2S (<10 mg m^{-3}), as defined by the Brazilian National Agency of Petroleum, Natural Gas, and Biofuels (ANP) [271].

Table S6 shows estimates on the potential production and application of bioCH_4 in Brazilian sugarcane biorefineries. Souza et al. [268] indicated that the bioCH_4 obtained from vinasse biodigestion in a large scale sugarcane biorefinery (8 MTC per season) could potentially supply up to 788 buses per day, with a daily autonomy of 400 km. Moraes et al. [63] reported that fossil fuel replacement could be the most economically attractive alternative for biogas in biorefineries when compared to the generation of electricity in engines and the cogeneration of electricity and steam in boilers. The total displacement of diesel, gasoline, and ethanol could reach 8000, 8900, and 11,500 m^3, respectively, in annexed or autonomous distilleries over a harvesting period of 167 days. No biogas upgrade was considered in this case. The results presented by Junqueira et al. [267] and Fuess and Zaiat [49] also indicate that the production of bioCH_4 from biogas–CH_4 in distilleries could lead to more favorable economic performances compared to the direct electricity generation in prime movers when considering greenfield and brownfield scenarios. Comparatively, the installation of prime movers is more expensive than the implementation of technological packages dedicated to biogas purification and distribution [49,270]. The replacement of diesel in agricultural operations and trucks directly reduces the production costs (both economic and environmental) of sugarcane. However, relatively significant infrastructure modifications are still necessary to manage eventually high bioCH_4 production levels, such as implementing gas grids nearby sugarcane facilities, and vice-versa. Conversely, the infrastructure required for managing electricity production is found in most sugarcane plants, in view of the sharing of equipment used in thermoelectricity (derived from bagasse) distribution.

The purification of biogas–CH_4 for bioCH_4 production also offers the opportunity for sulfur recovery in biorefineries (Figure 3) [3,192,272], depending on the technology used in the H_2S removal step. The desulfurization of biogas–CH_4 may be carried out according to various physical-chemical (e.g., in situ precipitation with iron salts; chemical adsorption in packed-bed columns, which may also use iron compounds or activated carbon as adsorbents; membrane separation; absorption using water or organic solvents) or biological (e.g., biofiltration; microaeration for H_2S oxidation; microalgae-based) processes [192,273,274]. Despite the technological consolidation of physical-chemical processes [274], the application of biological pathways for the upgrade of biogas–CH_4 offers important advantages, especially in terms of full-scale plants. Reaching equivalent removal efficiencies with reduced operating costs, eliminating chemical inputs and secondary residual streams (e.g., chemical sludge from H_2S precipitation with metallic salts), and potentially recovering bioresources, such as elemental sulfur and microalgae [192,273,275,276] exemplify the list of advantages. Among the upgrading options, the recovery of elemental sulfur in biorefineries could be obtained through the application of microaeration for the partial oxidation of H_2S (Equation (27)). In practical aspects, air (or pure oxygen) can be supplied either directly into the influent stream of the methanogenic reactors (liquid phase) or into the headspace of the systems. This technology is based on the activity of specific bacterial groups that grow lithoautotrophically on H_2S under limiting conditions of oxygen availability [273]. Consequently, sulfur reaches an intermediate oxidation state instead of being transformed into sulfate.

$$H_2S + 0.5O_2 \rightarrow S + H_2O \tag{27}$$

Experimental results from the monitoring of pilot-scale methanogenic reactors applied to sugarcane vinasse indicated H_2S concentrations in the biogas–CH_4 in the range of 0.5–2.2% v/v (5000–22,000 ppmv) [276,277]. Assuming the value of 256 g mol^{-1} for the molar mass of elemental sulfur [278], a H_2S concentration of 2.2% v/v in biogas (98.22 mol H_2S Nm^{-3} of biogas) could result in a theoretical elemental sulfur production of 25.14 kg S Nm^{-3} of biogas. Concerning economic aspects, gross earnings of 2.26 USD Nm^{-3} of biogas could be obtained by selling elemental sulfur, using a market price of 90.00 USD ton^{-1}S as a reference [279]. Using a very simple comparative approach (once prices are quoted in different periods), this value is at least 90% higher than the production costs estimated for bioCH_4, i.e., 0.95 USD Nm^{-3} [64], 0.30–0.31 USD Nm^{-3} [267], and 0.08–0.10 USD Nm^{-3} [49].

Elemental sulfur could also be blended with the biodigested vinasse and other residual streams from processing sugarcane to form an organomineral fertilizer (Figure 3), when considering some modifications in the system proposed by Gurgel et al. [132]. Junqueira et al. [267] also proposed the production of an organomineral fertilizer from biodigested sugarcane vinasse, which included a concentration step by evaporation (25°Brix) followed by the addition of ammonia up to a desired nitrogen–potassium ratio. In this system, the condensates recovered from the concentration step could be recycled as process water in different processes of the biorefinery, such as preparing yeast prior to fermentation. In particular, these authors reported the opportunity to incorporate relevant economic and environmental gains to the global sugarcane processing chain, primarily by reducing the inputs of diesel and mineral fertilizers, whose production chains also require high fossil fuel levels. Barrera et al. [272] highlighted that the sulfur recovered from biogass–CH_4 has a great potential for replacing the mineral sulfur supply provided by the inputs of ammonium sulfate in the cultivation of sugarcane vinasse.

5. Outlook: Implementing Biodigestion in Sugarcane Biorefineries

There are many opportunities for exploiting the recovery of bioresources from sugarcane vinasse based on the direct or indirect bioenergy generation from biogas and value-added metabolites obtained from the controlled bioconversion of organic matter. Regarding a preliminary (short-term) approach, the application of biodigestion as a robust technology for processing vinasse should be first disseminated in the Brazilian sugar and ethanol industry. Therefore, the production of biogas–CH_4 and its direct conversion into energy (instead of investing in the exploitation of fermentation products) should characterize the most immediate target of biodigestion in sugarcane biorefineries. Many favorable aspects could be observed, including [i] the technological maturity of large scale prime movers, such as engines and gas turbines, which combine high conversion efficiencies with operating robustness [280]; [ii] the suitability for recovering both electricity and thermal energy from biogas [280]; and [iii] the already existing infrastructure in biorefineries for exporting electricity to the grid. This approach requires a relatively simple infrastructure and has potential to promptly supply electricity demands, which are still much higher than the demands for bioCH_4 [270] or soluble products (from fermentation) in the Brazilian case.

The increasing relevance of bioenergy projects also favors the prompt implementation of full-scale biodigestion-power plants for generating electricity from biogas in biorefineries. In particular, the first full-scale project for electricity generation from biogas in the sucro-alcohol industry was approved in a public auction in Brazil just a few years ago (2016) for a thermoelectric plant with a 20.8 MW-capacity. The Brazilian Electricity Regulatory Agency (ANEEL) uses public auctions to purchase renewable energy from different sources in an attempt to encourage the production of bioenergy, decentralize the production of electricity exported to the grid, and diversify the Brazilian energy matrix [48]. Historically, projects related to using biogas have been absent from public auctions of renewable energy in Brazil, due to the higher profitability provided by other energy sources, such as wind, biomass, and bagasse [3]. However, financial incentives from the government, such as subsides, funding programs to reduce investment costs, and more restrictive environmental legislations, are still imperative to enable the implementation of full-scale biodigestion plants in biorefineries [3,49,184]. Although the lack of a national biogas program also affects the processing of wastewaters through biodigestion [184], the price paid by the electricity produced from the biogas in the aforementioned project (R$ 251.00 MWh^{-1} or USD 46.83 MWh^{-1}) was the highest value compared to other biofuels, such as sugarcane bagasse (R$ 181.25–245.20 MWh^{-1} or USD 33.82–45.75 MWh^{-1}) and wood chips (R$ 199.00–243.21 MWh^{-1} or USD 37.13–45.37 MWh^{-1}), which characterizes the bioenergy recovery from sugarcane vinasse as a promising approach.

The further diversification of alternatives for the exploitation of sugarcane vinasse will largely depend on the profile of each biorefinery in order to consider different aspects, which include: [i] the type of destination for the solid residues, i.e., bagasse, filter cake, and trash; [ii] the technological maturity of the alternative bioprocesses; and [iii] the location of the biorefinery relative to the natural gas grid. The cogeneration of energy from solid residues may favor adopting approaches for vinasse management that do not aim to directly recover energy from biogas, because electricity and steam obtained from bagasse tend to completely supply the requirements of the plants. However, this option does not eliminate the possibility of inserting biogas to boost the energy balance of the biorefineries, as detailed in Section 4.3. bioCH_4 production from biogas also characterizes a technology that could be promptly coupled with the implementation of vinasse biodigestion in biorefineries to focus on the replacement of diesel in transport operations. Although exporting bioCH_4 to the natural gas grid is an attractive option [49,267,270], the project may only be economically feasible in cases where the biorefinery is close to the grid. Distribution costs for long distances may be prohibitive because constructing networks for bioCH_4 distribution is costly [267].

Regardless of the predicted use for biogas, the operation of an independent acidogenic reactor characterizes a suitable option for full-scale biodigestion plants in biorefineries. Regarding short-term applications, the acidification of vinasse may be used exclusively for enhancing both the substrate biodegradability and bioenergy recovery through CH_4, as supported by experimental results [35,36]. The non-recovery of bioH_2 or other types of bioproducts from acidogenesis does not affect the economic feasibility of phase separation, as described by Fuess et al. [163,270]. In terms of medium- to long-term applications, the implemented acidogenic reactors could be modified to meet the requirements of the desired production process such that the extent of the modifications depends on the target bioproduct. For instance, the enhanced production of organic metabolites is likely to mainly require changes in operational parameters and chemical dosing, rather than installation aspects. In turn, additional reactors could be required when considering the production of biopolymers from the acidified vinasse, which also require the installation of aeration systems. Before any definitions, techno-economic assessments and scenarization-based approaches will be imperative to identify market opportunities. In contrast to the case of bioenergy production from biogas (Section 4.3.1), the literature is poor in terms of detailing techno-economic aspects of vinasse fermentation dedicated to the production and recovery of soluble metabolites. Nevertheless, it should be highlighted that the methanogenic step will fit into different stages of the global process, regardless of the type of bioresource recovered from the acidogenic phase (Figure 3).

Considering a broader analysis, it is worth noting that biodigestion has a great potential to be used as the core conversion step of the raw materials in biorefineries [270,281], instead of only processing residual streams (Figure 5). When focusing on the sugar and ethanol industry, important advantages could be observed by directly applying biodigestion to the processing of energy crops, i.e., sugarcane in this case:

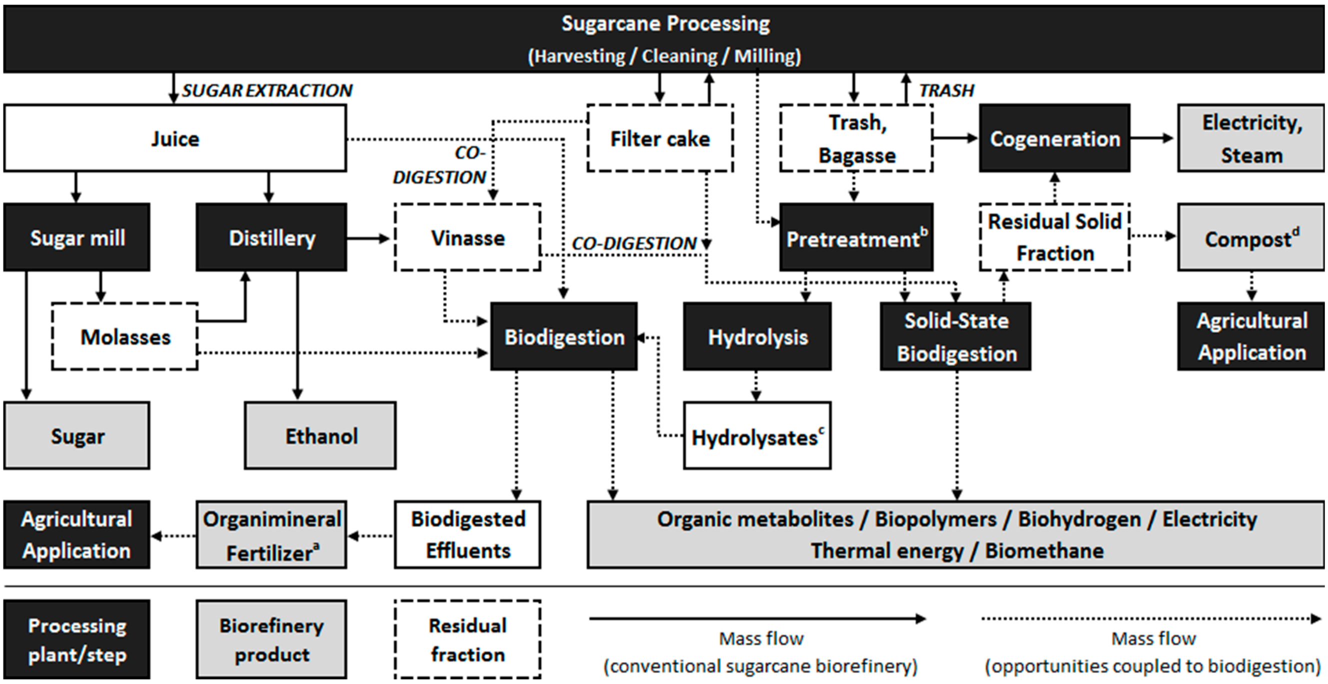

Figure 5. Opportunities for inserting biodigestion in 1G sugarcane biorefineries. Notes: [a] Requires the post-treatment of the effluent, such as concentration, blending with other residual streams (e.g., filter cake), and mineral supplementation. [b] Aims to reduce the recalcitrance of the lignocellulosic materials. [c] Composed of pentoses and hexoses. [d] Requires the post-treatment of the digestate, such as mineral supplementation.

1. Ethanol plants are capital intensive and require several unit processes (e.g., fermentation, centrifugation, and distillation) that demand high levels of energy, while biodigestion requires much less investment and energy inputs [46,269]. Fuess et al. [50] estimated that the installation costs of biodigestion systems coupled with power plants for recovering bioenergy from vinasse would be less than 10% of the total investment with large scale sugarcane biorefineries (with milling capacities of 4 MTC and 1.7 MTC in the harvesting and inter-harvesting periods, respectively). This particular analysis already considers the installation of two-phase anaerobic plants. Similarly, Junqueira et al. [267] also indicated that the biodigestion of vinasse coupled with the recovery of bioenergy accounts for a minimal fraction of the total investment in sugarcane biorefineries, and ranges from 3.4–7.5% depending on the technological package considered for exploiting the biogas–CH_4 (cogeneration of electricity and steam in boilers, electricity generation in engines, bioCH_4 production for diesel replacement and bioCH_4 production, and further injection into the gas grid).
2. The energy potential of 1 ton of cleaned sugarcane is approximately 1718 MCal, which is characterized by the fractions of sugars (153 kg, 608 Mcal), bagasse with 50% moisture (216 kg, 598 Mcal), and trash with 15% moisture (165 kg, 512 Mcal) [282]. Thus, the maximum theoretical energy recoverable from sugarcane through 1G ethanol production could reach roughly 35%, when assuming the conversion of the total fraction of sugars and without taking into consideration energy losses due to parallel metabolic pathways and yeast growth. However, in practical aspects, the ethanol productivity (in which all "metabolic losses" are included) currently obtained in 1G sugarcane biorefineries (82 L TC^{-1} [5]) leads to an effective energy recovery below 25% of the total energy in sugarcane (416 Mcal) when considering a LHV of 21.22 MJ L^{-1} for ethanol [62]. Conversely, van Haandel and Catunda [46] and Wilkie [269] indicated that biodigestion may lead to a more efficient use of the sugars present in sugarcane. The direct conversion of the juice into biogas–CH_4 would recover approximately 50% of the total energy present in the sugarcane. Fuess et al. [281] associated an

energy return on investment (EROI) ratio to the direct biogas production (and further conversion into electricity) from juice and juice/bagasse as four times higher than the one observed in ethanol production. In practical terms, the results indicate that producing energy from biogas is more efficient than using ethanol, which fully suits one of the UN Sustainable Development Goals (#7: Ensure access to affordable, reliable, sustainable, and modern energy for all [283]). The further conversion of bagasse into biogas in anaerobic systems, either via solid-state biodigestion [284,285] or via sugar-rich hydrolisates [10–12,286] (Figure 5), could enhance the energy recovery to 60–80% [46,269], while the integration of 1G and 2G sugarcane biorefineries may increase the energy recovery through ethanol production to only 30–34% (i.e., 518–589 Mcal, considering global ethanol productivities in the range of 102–116 L TC^{-1} [5]). Some energy recovery improvements in 2G ethanol plants could be achieved through the biodigestion of 2G vinasse and pentose liquor (HSW streams depicted in Figure 1b), as proposed elsewhere [66].

3. The better performance of biodigestion compared to yeast-driven fermentation in terms of energy extraction directly affects the energy potential of the produced biofuels, because the LHV of CH_4 (50.00 MJ kg^{-1} [223]) is approximately double the value observed for ethanol (26.90 MJ kg^{-1} [223]). Moreover, biogas applications (either purified or non-purified) are diverse (Figure 5), insofar as the layout of the biorefineries could be designed according to the most profitable option, i.e., the production of biochemicals (through acidogenesis), electricity generation from biogas–CH_4, and bioCH_4 production. In practical terms, biogas-based biorefineries are much more flexible than those producing ethanol as the main biofuel [270,281], which fully suits the biorefining concept presented elsewhere [41]: "the sustainable processing of biomass into a spectrum of marketable products, which means: energy, materials, chemicals, food and feed". Naturally, the implementation of sugarcane biorefineries using biodigestion as the core process does not exclude industrial plants based on the traditional production of sugar and/or ethanol, in which the biodigestion of vinasse should necessarily be incorporated. In short, the aforementioned aspects could be carefully considered in future greenfield projects of the sugar and ethanol sector to consider biodigestion as an efficient step for exploiting sugarcane, either directly or from the residual fractions.

Finally, an equally huge amount of opportunities results from exploiting the co-digestion of sugarcane-derived residues, as substantially studied from the middle of the last decade. For instance, numerous experimental studies, usually dealing with the co-processing of vinasse and filter cake, are currently available [8,13,287,288]. There are also studies addressing techno-economic analysis of biogas use in such co-digestion-based alternatives [192,289]. Thus, a successful future is expected for biodigestion within the context of sugarcane processing plants, as also motivated by the creation of the Brazilian National Biofuel Policy (RenovaBio) and other correlated policy acts, in which biofuel production from biodigestion plants is one of the key aspects considered to diversify the Brazilian energy matrix. Additional references [290–292] are found in Table S2, while references [293–295] are cited in Tables S3 and S4, respectively.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomass2040025/s1, Table S1: Compositional characterization of sugarcane vinasses from juice and blends of juice and molasses [43,44,60,77–79,82,88,211,219–222]; Table S2: Alternative (bio)technological applications proposed for sugarcane vinasse [54–57,73,76,102–109,111–114,126,127,132–138,174,290–292]; Table S3: Studies on the biodigestion of sugarcane vinasse in Brazil [33–38,59,60,174–177,293,294]; Table S4: Estimates of the energetic potential of sugarcane vinasse targeting electricity production from biogas–CH_4 [46,48–50,52,61–66,78,184,186,187,189–192,295]; Table S5: Studies on the production of bio-H_2 from sugarcane vinasse in Brazil [44,77,79,208–215,217–222,294]; Table S6: Estimates of the potential production and application of bio-CH_4 in Brazilian sugarcane biorefineries [49,63,192,267,268].

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References

1. Lima, M.A.P.; Bonomi, A.; Cavalett, O.; Cunha, M.P. Background. In *Virtual Biorefinery: An Optimization Strategy for Renewable Carbon Valorization*; Bonomi, A., Cavalett, O., Cunha, M.P., Lima, M.A.P., Eds.; Springer: London, UK, 2016; pp. 1–4.
2. Dias, M.O.S.; Modesto, M.; Ensinas, A.V.; Nebra, S.A.; Maciel Filho, R.; Rossell, C.E.V. Improving bioethanol production from sugarcane: Evaluation of distillation, thermal integration and cogeneration systems. *Energy* **2011**, *36*, 3691–3703. [CrossRef]
3. Moraes, B.S.; Zaiat, M.; Bonomi, A. Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: Challenges and perspectives. *Renew. Sustain. Energy Rev.* **2015**, *44*, 888–903. [CrossRef]
4. Junqueira, T.L.; Cavalett, O.; Bonomi, A. The virtual sugarcane biorefinery—A simulation tool to support public policies formulation in bioenergy. *Ind. Biotechnol.* **2016**, *12*, 62–67. [CrossRef]
5. Dias, M.O.S.; Junqueira, T.L.; Cavalett, O.; Cunha, M.P.; Jesus, C.D.F.; Rossell, C.E.V.; Maciel Filho, R.; Bonomi, A. Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. *Bioresour. Technol.* **2012**, *103*, 152–161. [CrossRef] [PubMed]
6. Dias, M.O.S.; Junqueira, T.L.; Cavalett, O.; Pavanello, L.G.; Cunha, M.P.; Jesus, C.D.F.; Maciel Filho, R.; Bonomi, A. Biorefineries for the production of first and second generation ethanol and electricity from sugarcane. *Appl. Energy* **2013**, *109*, 72–78. [CrossRef]
7. Morais, E.R.; Junqueira, T.L.; Sampaio, I.L.M.; Dias, M.O.S.; Rezende, M.C.A.F.; Jesus, C.D.F.; Klein, B.C.; Gómez, E.O.; Mantelatto, P.E.; Maciel Filho, R.; et al. Biorefinery alternatives. In *Virtual Biorefinery: An Optimization Strategy for Renewable Carbon Valorization*; Bonomi, A., Cavalett, O., Cunha, M.P., Lima, M.A.P., Eds.; Springer: London, UK, 2016; pp. 53–132.
8. Volpi, M.P.C.; Ferraz, A.D.N., Jr.; Franco, T.T.; Moraes, B.S. Operational and biochemical aspects of co-digestion (co-AD) from sugarcane vinasse, filter cake, and deacetylation liquor. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 8969–8987. [CrossRef]
9. Lora, E.E.S.; Andrade, R.V.; Ángel, J.D.M.; Leite, M.A.H.; Rocha, M.H.; Sales, C.A.V.B.; Mendoza, M.A.G.; Coral, D.S.O. Gasification and pyrolysis for converting biomass into electricity and biofuels. In *Biofuels*; Lora, E.E.S., Venturini, O.J., Eds.; Interciência: Rio de Janeiro, Brazil, 2012; Volume 1, pp. 411–498.
10. Baêta, B.E.L.; Lima, D.R.S.; Balena Filho, J.G.; Adarme, O.F.H.; Gurgel, L.V.A.; Aquino, S.F. Evaluation of hydrogen and methane production from sugarcane bagasse hemicellulose hydrolysates by two-stage anaerobic digestion process. *Bioresour. Technol.* **2016**, *218*, 436–446. [CrossRef]
11. Bolado-Rodríguez, S.; Toquero, C.; Martín-Juárez, J.; Travaini, R.; García-Encina, P.A. Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. *Bioresour. Technol.* **2016**, *201*, 182–190. [CrossRef]
12. Jafari, O.; Zilouei, H. Enhanced biohydrogen and subsequent biomethane production from sugarcane bagasse using nano-titanium dioxide pretreatment. *Bioresour. Technol.* **2016**, *214*, 670–678. [CrossRef]
13. Janke, L.; Weinrich, S.; Leite, A.F.; Sträuber, H.; Radetski, C.M.; Nikolausz, M.; Nelles, M.; Stinner, W. Year-round biogas production in sugarcane biorefineries: Process stability, optimization and performance of a two-stage reactor system. *Energy Convers. Manag.* **2018**, *168*, 188–199. [CrossRef]
14. Almeida, A.B., Jr.; Nascimento, C.W.A.; Sobral, M.F.; Silva, F.B.V.; Gomes, W.A. Soil fertility and uptake of nutrients by sugarcane fertilized with filter cake. *Rev. Bras. Eng. Agríc. Ambient.* **2011**, *15*, 1004–1013. (In Portuguese)
15. Contreras, A.M.; Rosa, E.; Pérez, M.; van Langenhove, H.; Dewulf, J. Comparative life cycle assessment of four alternatives for using by-products of cane sugar production. *J. Clean. Prod.* **2009**, *17*, 772–779. [CrossRef]
16. España-Gamboa, E.; Mijangos-Cortes, J.; Barahona-Perez, L.; Dominguez-Maldonado, J.; Hernández-Zarate, G.; Alzate-Gaviria, L. Vinasses: Characterization and treatments. *Waste Manag. Res.* **2011**, *29*, 1235–1250. [CrossRef] [PubMed]
17. Fuess, L.T.; Garcia, M.L. Implications of stillage land disposal: A critical review on the impacts of fertigation. *J. Environ. Manag.* **2014**, *145*, 210–229. [CrossRef]
18. Fuess, L.T.; Altoé, M.E.; Felipe, M.C.; Garcia, M.L. Pros and cons of fertirrigation with *in natura* sugarcane vinasse: Do improvements in soil fertility offset environmental and bioenergy losses? *J. Clean. Prod.* **2021**, *319*, 128684. [CrossRef]
19. Dias, M.O.S.; Maciel Filho, R.; Mantelatto, P.E.; Cavalett, O.; Rossell, C.E.V.; Bonomi, A.; Leal, M.R.L.V. Sugarcane processing for ethanol and sugar in Brazil. *Environ. Dev.* **2015**, *15*, 35–51. [CrossRef]
20. Oliveira, B.G.; Carvalho, J.L.N.; Cerri, C.E.P.; Cerri, C.C.; Feigl, B.J. Soil greenhouse gas fluxes from vinasse application in Brazilian sugarcane areas. *Geoderma* **2013**, *200–201*, 77–84. [CrossRef]

21. Carvalho, J.L.; Oliveira, B.G.; Cantarella, H.; Chagas, M.F.; Gonzaga, L.C.; Lourenço, K.S.; Bordonal, R.O.; Bonomi, A. Implications of regional N_2O-N emission factors on sugarcane ethanol emissions and granted decarbonization certificates. *Renew. Sustain. Energy Rev.* **2021**, *149*, 111423. [CrossRef]
22. Oliveira, B.G.; Lourenço, K.S.; Carvalho, J.L.N.; Gonzaga, L.C.; Teixeira, M.C.; Tamara, A.F.; Cantarella, H. Soil pH does not interfere with nitrification inhibitor efficiency for reducing N_2O emissions from soils treated with concentrated vinasse and urea. *Geoderma* **2022**, *426*, 116087. [CrossRef]
23. Wilkie, A.C.; Riedesel, K.J.; Owens, J.M. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass Bioenergy* **2000**, *19*, 63–102. [CrossRef]
24. Rodrigues, I.J.; Fuess, L.T.; Biondo, L.; Santesso, C.A.; Garcia, M.L. Coagulation-flocculation of anaerobically treated sugarcane stillage. *Desalin. Water Treat.* **2014**, *52*, 4111–4121. [CrossRef]
25. Guerreiro, L.F.; Rodrigues, C.S.D.; Duda, R.M.; Oliveira, R.A.; Boaventura, R.A.R.; Madeira, L.M. Treatment of sugarcane vinasse by combination of coagulation/flocculation and Fenton's oxidation. *J. Environ. Manag.* **2016**, *181*, 237–248. [CrossRef] [PubMed]
26. Zayas, T.; Romero, V.; Salgado, L.; Meraz, M.; Morales, U. Applicability of coagulation/flocculation and electrochemical processes to the purification of biologically treated vinasse effluent. *Sep. Purif. Technol.* **2007**, *57*, 270–276. [CrossRef]
27. Seixas, F.L.; Gimenes, M.L.; Fernades-Machado, N.R.C. Treatment of vinasse by adsorption on carbon from sugar cane bagasse. *Quim. Nova* **2016**, *39*, 172–179. (In Portuguese) [CrossRef]
28. Aquino, S.; Pires, E.C. Assessment of ozone as a pretreatment to improve anaerobic digestion of vinasse. *Braz. J. Chem. Eng.* **2016**, *33*, 279–285. [CrossRef]
29. Asaithambi, P.; Susree, M.; Saravanathamizhan, R.; Matheswaran, M. Ozone assisted electrocoagulation for the treatment of distillery effluent. *Desalination* **2012**, *297*, 1–7. [CrossRef]
30. Yavuz, Y. EC and EF processes for the treatment of alcohol distillery wastewater. *Sep. Purif. Technol.* **2007**, *53*, 135–140. [CrossRef]
31. de Bazúa, C.D.; Cabrero, M.A.; Poggi, H.M. Vinasses biological treatment by anaerobic and aerobic processes: Laboratory and pilot-plant tests. *Bioresour. Technol.* **1991**, *35*, 87–93. [CrossRef]
32. Ferreira, L.F.R.; Aguiar, M.M.; Messias, T.G.; Pompeu, G.B.; Lopez, A.M.Q.; Silva, D.P.; Monteiro, R.T. Evaluation of sugar-cane vinasse treated with *Pleurotus sajor-caju* utilizing aquatic organisms as toxicological indicators. *Ecotox. Environ. Saf.* **2011**, *74*, 132–137. [CrossRef]
33. Aquino, S.; Fuess, L.T.; Pires, E.C. Media arrangement impacts cell growth in anaerobic fixed-bed reactors treating sugarcane vinasse: Structured vs. randomic biomass immobilization. *Bioresour. Technol.* **2017**, *235*, 219–228. [CrossRef]
34. Craveiro, A.M.; Soares, H.M.; Schmidell, W. Technical aspects and cost estimations for anaerobic systems treating vinasse and brewery/soft drink wastewaters. *Water Sci. Technol.* **1986**, *18*, 123–134. [CrossRef]
35. Ferraz, A.D.N., Jr.; Koyama, M.H.; Araújo, M.M., Jr.; Zaiat, M. Thermophilic anaerobic digestion of raw sugarcane vinasse. *Renew. Energy* **2016**, *89*, 245–252.
36. Fuess, L.T.; Kiyuna, L.S.M.; Ferraz, A.D.N., Jr.; Persinoti, G.F.; Squina, F.M.; Garcia, M.L.; Zaiat, M. Thermophilic two-phase anaerobic digestion using innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse. *Appl. Energy* **2017**, *189*, 480–491. [CrossRef]
37. Souza, M.E.; Fuzaro, G.; Polegato, A.R. Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. *Water Sci. Technol.* **1992**, *25*, 213–222. [CrossRef]
38. Del Nery, V.; Alves, I.; Damianovic, M.H.R.Z.; Pires, E.C. Hydraulic and organic rates applied to pilot scale UASB reactor for sugar cane vinasse degradation and biogas generation. *Biomass Bioenergy* **2018**, *119*, 411–417. [CrossRef]
39. Ramos, L.R.; Lovato, G.; Rodrigues, J.A.D.; Silva, E.L. Anaerobic digestion of vinasse in fluidized bed reactors: Process robustness between two-stage thermophilic-thermophilic and thermophilic-mesophillic systems. *J. Clean. Prod.* **2021**, *314*, 128066. [CrossRef]
40. Barbosa, M.Y.U.; Alves, I.; Del Nery, V.; Sakamoto, I.K.; Pozzi, E.; Damianovic, M.H.R.Z. Methane production in a UASB reactor from sugarcane vinasse: Shutdown or exchanging substrate for molasses during the off-season? *J. Water Process Eng.* **2022**, *47*, 102664. [CrossRef]
41. Poggi-Varaldo, H.M.; Munoz-Paez, K.M.; Escamilla-Alvarado, C.; Robledo-Narváez, P.N.; Ponce-Noyola, M.T.; Calva-Calva, G.; Ríos-Leal, E.; Galíndez-Mayer, J.; Estrada-Vázquez, C.; Ortega-Clemente, A.; et al. Biohydrogen, biomethane and bioelectricity as crucial components of biorefinery of organic wastes: A review. *Waste Manag. Res.* **2014**, *32*, 353–365. [CrossRef] [PubMed]
42. Yang, X.; Choi, H.S.; Park, C.; Kim, S.W. Current states and prospects of organic waste utilization for biorefineries. *Renew. Sustain. Energy Rev.* **2015**, *49*, 335–349. [CrossRef]
43. Sánchez, F.E.; Fuess, L.T.; Cavalcante, G.S.; Adorno, M.A.T.; Zaiat, M. Value-added soluble metabolite production from sugarcane vinasse within the carboxylate platform: An application of the anaerobic biorefinery beyond biogas production. *Fuel* **2021**, *286*, 119378. [CrossRef]
44. Fuess, L.T.; Kiyuna, L.S.M.; Garcia, M.L.; Zaiat, M. Operational strategies for long-term biohydrogen production from sugarcane stillage in continuous acidogenic packed-bed reactor. *Int. J. Hydrogen Energy* **2016**, *41*, 8132–8145. [CrossRef]
45. Siles, J.A.; García-García, I.; Martín, A.; Martín, M.A. Integrated ozonation and biomethanization treatments of vinasse derived from ethanol manufacturing. *J. Hazard. Mater.* **2011**, *188*, 247–253. [CrossRef] [PubMed]
46. van Haandel, A.C.; Catunda, P.F.C. Profitability increase of alcohol distilleries by the rational use of byproducts. *Water Sci. Technol.* **1994**, *29*, 117–124. [CrossRef]

47. Willington, I.P.; Marten, G.G. Options for handling stillage waste from sugar-based fuel ethanol production. *Resour. Conserv.* **1982**, *8*, 111–129. [CrossRef]
48. Fuess, L.T.; Garcia, M.L. Bioenergy from stillage anaerobic digestion to enhance the energy balance ratio of ethanol production. *J. Environ. Manag.* **2015**, *162*, 102–114. [CrossRef]
49. Fuess, L.T.; Zaiat, M. Economics of anaerobic digestion for processing sugarcane vinasse: Applying sensitivity analysis to increase process profitability in diversified biogas applications. *Process Saf. Environ. Prot.* **2018**, *115*, 27–37. [CrossRef]
50. Fuess, L.T.; Klein, B.C.; Chagas, M.F.; Rezende, M.C.A.F.; Garcia, M.L.; Bonomi, A.; Zaiat, M. Diversifying the technological strategies for recovering bioenergy from the two-phase anaerobic digestion of sugarcane vinasse: An integrated techno-economic and environmental approach. *Renew. Energy* **2018**, *122*, 674–687. [CrossRef]
51. Ramos, L.R.; Lovato, G.; Rodrigues, J.A.D.; Silva, E.L. Scale-up and energy estimations of single- and two-stage vinasse anaerobic digestion systems for hydrogen and methane production. *J. Clean. Prod.* **2022**, *349*, 131459. [CrossRef]
52. Borges, A.V.; Fuess, L.T.; Alves, I.; Takeda, P.Y.; Damianovic, M.H.R.Z. Co-digesting sugarcane vinasse and distilled glycerol to enhance bioenergy generation in biofuel-producing plants. *Energy Convers. Manag.* **2021**, *250*, 114897. [CrossRef]
53. Takeda, P.Y.; Oliveira, C.A.; Dias, M.E.S.; Paula, C.T.; Borges, A.V.; Damianovic, M.H.R.Z. Enhancing the energetic potential of sugarcane biorefinery exchanging vinasse and glycerol in sugarcane off-season in an anaerobic reactor. *Renew. Energy* **2022**, *195*, 1218–1229. [CrossRef]
54. Aguiar, M.M.; Ferreira, L.F.R.; Monteiro, R.T.R. Use of vinasse and sugarcane bagasse for the production of enzymes by lignocellulolytic fungi. *Braz. Arch. Biol. Technol.* **2010**, *53*, 1245–1254. [CrossRef]
55. Oliveira, J.G.; Garcia-Cruz, C.H. Properties of a biosurfactant produced by *Bacillus pumilus* using vinasse and waste frying oil as alternative carbon sources. *Braz. Arch. Biol. Technol.* **2013**, *56*, 155–160. [CrossRef]
56. Nitayavardhana, S.; Khanal, S.K. Innovative biorefinery concept for sugar-based ethanol industries: Production of protein-rich fungal biomass on vinasse as an aquaculture feed ingredient. *Bioresour. Technol.* **2010**, *101*, 9078–9085. [CrossRef] [PubMed]
57. Ramirez, N.N.V.; Farenzena, M.; Trierweiler, J.O. Growth of microalgae *Scenedesmus* sp. in ethanol vinasse. *Braz. Arch. Biol. Technol.* **2014**, *57*, 630–635. [CrossRef]
58. Siqueira, J.C.; Braga, M.Q.; Ázara, M.S.; Garcia, K.J.; Alencar, S.N.M.; Ramos, T.S.; Siniscalchi, L.A.B.; Assemany, P.P.; Ensinas, A.V. Recovery of vinasse with combined microalgae cultivation in a conceptual energy-efficient industrial plant: Analysis of related process considerations. *Renew. Sustain. Energy Rev.* **2022**, *155*, 111904. [CrossRef]
59. Döll, M.M.R.; Foresti, E. Effect of the sodium bicarbonate in the treatment of vinasse in AnSBBR operated at 55 and 35 °C. *Eng. Sanit. Ambient.* **2010**, *15*, 275–282. (In Portuguese) [CrossRef]
60. Siqueira, L.M.; Damiano, E.S.G.; Silva, E.L. Influence of organic loading rate on the anaerobic treatment of sugarcane vinasse and biogas production in fluidized bed reactor. *J. Environ. Sci. Health A* **2013**, *48*, 1707–1716. [CrossRef]
61. Albanez, R.; Chiaranda, B.C.; Ferreira, R.G.; França, A.L.P.; Honório, C.D.; Rodrigues, J.A.D.; Ratusznei, S.M.; Zaiat, M. Anaerobic biological treatment of vinasse for environmental compliance and methane production. *Appl. Biochem. Biotechnol.* **2016**, *178*, 21–43. [CrossRef]
62. Fuess, L.T.; Garcia, M.L. Anaerobic digestion of stillage to produce bioenergy in the sugarcane-to-ethanol industry. *Environ. Technol.* **2014**, *35*, 333–339. [CrossRef]
63. Moraes, B.S.; Junqueira, T.L.; Pavanello, L.G.; Cavalett, O.; Mantelatto, P.E.; Bonomi, A.; Zaiat, M. Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: Profit or expense? *Appl. Energy* **2014**, *113*, 825–835. [CrossRef]
64. Salomon, K.R.; Lora, E.E.S.; Rocha, M.H.; del Olmo, O.A. Cost calculations for biogas from vinasse biodigestion and its energy utilization. *Sugar Ind.* **2011**, *136*, 217–223. [CrossRef]
65. van Haandel, A.C. Integrated energy production and reduction of the environmental impact at alcohol distillery plants. *Water Sci. Technol.* **2005**, *52*, 49–57. [CrossRef] [PubMed]
66. Longati, A.A.; Lino, A.R.A.; Giordano, R.C.; Furlan, F.F.; Cruz, A.J.G. Biogas production from anaerobic digestion of vinasse in sugarcane biorefinery: A techno-economic and environmental analysis. *Waste Biomass Valor.* **2020**, *11*, 4573–4591. [CrossRef]
67. Ghimire, A.; Frunzo, L.; Pirozzi, F.; Trably, E.; Escudie, R.; Lens, P.N.L.; Esposito, G. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. *Appl. Energy* **2015**, *144*, 73–95. [CrossRef]
68. Oliveira, G.H.D.; Zaiat, M.; Rodrigues, J.A.D.; Ramsay, J.A.; Ramsay, B.A. Towards the production of mcl-PHA with enriched dominant monomer content: Process development for the sugarcane biorefinery context. *J. Polym. Environ.* **2020**, *28*, 844–853. [CrossRef]
69. Pacheco, J.M.; Hoff, D.N. Closing the matter and energy cycle in the sugar/alcohol sector. *Sustain. Debate* **2013**, *4*, 215–236. (In Portuguese) [CrossRef]
70. Christofoletti, C.A.; Escher, J.P.; Correia, J.E.; Marinho, J.F.U.; Fontanetti, C.S. Sugarcane vinasse: Environmental implications of its use. *Waste Manag.* **2013**, *33*, 2752–2761. [CrossRef] [PubMed]
71. Sheehan, G.J.; Greenfield, P.F. Utilisation, treatment and disposal of distillery wastewater. *Water Res.* **1980**, *14*, 257–277. [CrossRef]
72. Dowd, M.K.; Johansen, S.L.; Cantarella, L.; Reilly, P.J. Low molecular weight organic composition of ethanol stillage from sugarcane molasses, citrus waste, and sweet whey. *J. Agric. Food Chem.* **1994**, *42*, 283–288. [CrossRef]
73. Navarro, A.R.; Sepúlveda, M.C.; Rubio, M.C. Bio-concentration of vinasse from the alcoholic fermentation of sugar cane molasses. *Waste Manag.* **2000**, *20*, 581–585. [CrossRef]

74. Melo, H.F. Response to Acid Stress in Industrial Fermentation Yeasts. Ph.D. Thesis, Federal University of Pernambuco, Recife, Brazil, 2006. (In Portuguese)
75. Pereira, I.M. Study of Some Parameters for Glycerol Precipitation by Complexation with Zirconium in Vinasses from Fuel Alcohol and Spirits and Weak Leaches of Soap Manufacture. Master's Thesis, University of Campinas, Campinas, Brazil, 1991. (In Portuguese)
76. Bonini, M.A. Heterotrophic Cultivation of *Aphanothece microscopica Nägeli* and *Chlorella vulgaris* in Different Carbon Sources and Vinasse. Master's Thesis, Federal University of São Carlos, Araras, Brazil, 2012. (In Portuguese)
77. Fuess, L.T.; Zaiat, M.; Nascimento, C.A.O. Novel insights on the versatility of biohydrogen production from sugarcane vinasse via thermophilic dark fermentation: Impacts of pH-driven operating strategies on acidogenesis metabolite profiles. *Bioresour. Technol.* **2019**, *286*, 121379. [CrossRef] [PubMed]
78. Santos, P.S.; Zaiat, M.; Nascimento, C.A.O.; Fuess, L.T. Does sugarcane vinasse composition variability affect the bioenergy yield in anaerobic systems? A dual kinetic-energetic assessment. *J. Clean. Prod.* **2019**, *240*, 118005. [CrossRef]
79. Piffer, M.A.; Zaiat, M.; Nascimento, C.A.O.; Fuess, L.T. Dynamics of sulfate reduction in the thermophilic dark fermentation of sugarcane vinasse: A biohydrogen-independent approach targeting enhanced bioenergy production. *J. Environ. Chem. Eng.* **2021**, *9*, 105956. [CrossRef]
80. Borges, A.V.; Fuess, L.T.; Takeda, P.Y.; Alves, I.; Dias, M.E.S.; Damianovic, M.H.R.Z. Co-digestion of biofuel by-products: Enhanced biofilm formation maintains high organic matter removal when methanogenesis fails. *J. Environ. Manag.* **2022**, *310*, 114768. [CrossRef] [PubMed]
81. Fuess, L.T.; Piffer, M.A.; Zaiat, M.; Nascimento, C.A.O. Phase separation enhances bioenergy recovery in sugarcane vinasse biodigestion: Absolute or relative truth? *Bioresour. Technol. Rep.* **2022**, *18*, 101026. [CrossRef]
82. Moraes, B.S.; Santos, G.M.; Delforno, T.P.; Fuess, L.T.; Silva, A.J. Enriched microbial consortia for dark fermentation of sugarcane vinasse towards value-added short-chain organic acids and alcohol production. *J. Biosci. Boeng.* **2019**, *127*, 594–601. [CrossRef]
83. Serafim, F.A.T.; Buchviser, S.F.; Galinaro, C.A.; Franco, D.W.; Novaes, F.V. Organic acids in sugarcane spirits' fractions produced in stills and columns. *Quim. Nova* **2011**, *34*, 28–32. (In Portuguese) [CrossRef]
84. Basso, C.J.; Santi, A.L.; Lamego, F.P.; Somavilla, L.; Brigo, T.J. Vinasse as a source of potassium: Black oat/corn silage/short-season corn succession response and soil chemical alterations in the Northwest region of the state of Rio Grande do Sul. *Cienc. Rural* **2013**, *43*, 596–602. (In Portuguese) [CrossRef]
85. Barth, D.; Monteiro, A.R.S.; Costa, M.M.; Virkajärvi, I.; Sacon, V.; Wilhelmsom, A. DesinFix TM 135 in fermentation process for bioethanol production. *Braz. J. Microbiol.* **2014**, *45*, 323–325. [CrossRef]
86. Ceccato-Antonini, S.R. Conventional and nonconventional strategies for controlling bacterial contamination in fuel ethanol fermentations. *World J. Microbiol. Biotechnol.* **2018**, *34*, 80. [CrossRef]
87. Kiyuna, L.S.M.; Fuess, L.T.; Zaiat, M. Unraveling the influence of the COD/sulfate ratio on organic matter removal and methane production from the biodigestion of sugarcane vinasse. *Bioresour. Technol.* **2017**, *232*, 103–112. [CrossRef] [PubMed]
88. Fuess, L.T.; Rodrigues, I.J.; Garcia, M.L. Fertirrigation with sugarcane vinasse: Foreseeing potential impacts on soil and water resources through vinasse characterization. *J. Environ. Sci. Health A* **2017**, *52*, 1063–1072. [CrossRef] [PubMed]
89. Brito, F.L.; Rolim, M.M.; Silva, J.A.A.; Pedrosa, E.M.R. Quality of percolate from soils after application of different doses and incubation time of vinasse. *Rev. Bras. Eng. Agríc. Ambient.* **2007**, *11*, 318–323. (In Portuguese) [CrossRef]
90. Brito, F.L.; Rolim, M.M.; Pedrosa, E.M.R. Effect of vinasse application in chemical characteristics of soils from forest zone of Pernambuco. *Rev. Bras. Ciênc. Agrár.* **2009**, *4*, 456–462. (In Portuguese)
91. Camilotti, F.; Marques, M.O.; Andrioli, I.; Silva, A.R.; Tasso, L.C., Jr.; Nobile, F.O. Heavy metals accumulation in sugarcane after application in sewage sludge and vinasse. *Eng. Agríc.* **2007**, *27*, 284–293. (In Portuguese) [CrossRef]
92. Gunkel, G.; Kosmol, J.; Sobral, M.; Rohn, H.; Montenegro, S.; Aureliano, J. Sugar cane industry as a source of water pollution—Case study on the situation in Ipojuca river, Pernambuco, Brazil. *Water Air Soil Poll.* **2007**, *180*, 261–269. [CrossRef]
93. Lyra, M.R.C.C.; Rolim, M.M.; Silva, J.A.A. Toposequence of soils fertigated with stillage: Contribution towards the quality of ground water table. *Rev. Bras. Eng. Agríc. Ambient.* **2003**, *7*, 525–532. (In Portuguese) [CrossRef]
94. Miranda, T.L.; Pedrosa, E.M.R.; Silva, E.F.F.; Rolim, M.M. Physical and biological alterations in sugarcane cultivated soil after harvest and vinasse application. *Rev. Bras. Ciênc. Agrár.* **2012**, *7*, 150–158. (In Portuguese)
95. Moore, C.C.S.; Nogueira, A.R.; Kulay, L. Environmental and energy assessment of the substitution of chemical fertilizers for industrial wastes of ethanol production in sugarcane cultivation in Brazil. *Int. J. Life Cycle Assess.* **2017**, *22*, 628–643. [CrossRef]
96. Ribeiro, B.T.; Lima, J.M.; Guilherme, L.R.G.; Julião, L.G.F. Lead sorption and leaching from an Inceptisol sample amended with sugarcane vinasse. *Sci. Agric.* **2010**, *67*, 441–447. [CrossRef]
97. Rolim, M.M.; Lyra, M.R.C.C.; Duarte, A.S.; Medeiros, P.R.F.; França e Silva, E.F.; Pedrosa, E.M.R. Influence of a vinasse-distributing lake on water quality of the groundwater. *Rev. Ambient. Água* **2013**, *8*, 155–171. (In Portuguese) [CrossRef]
98. Silva, W.P.; Almeida, C.D.G.C.; Rolim, M.M.; Silva, E.F.F.; Pedrosa, E.M.R.; Silva, V.G.F. Monitoring of groundwater salinity in lowland under sugarcane cultivation fertigated with vinasse. *Rev. Bras. Eng. Agríc. Ambient.* **2014**, *18*, 394–401. (In Portuguese) [CrossRef]
99. Tasso, L.G., Jr.; Marques, M.O.; Franco, A.; Nogueira, G.A.; Nobile, F.O.; Camilotti, F.; Silvam, A.R. Yield and quality of sugar cane cultivated in sewage sludge; vinasse and mineral fertilization supplied soil. *Eng. Agríc.* **2007**, *27*, 276–283. (In Portuguese)

100. Uyeda, C.A.; Miranda, J.H.; Duarte, S.N.; Medeiros, P.R.F.; Dias, C.T.S. Influence of vinasse application in hydraulic conductivity of three soils. *Eng. Agríc.* **2013**, *33*, 689–698. [CrossRef]
101. Zolin, C.A.; Paulino, J.; Bertonha, A.; Freitas, P.S.L.; Folegatti, M.V. Exploratory study of the stillage use along the time. I. Characteristics of the soil. *Rev. Bras. Eng. Agríc. Ambient.* **2011**, *15*, 22–28. (In Portuguese) [CrossRef]
102. Kadioğlu, A.; Algur, O.F. Tests of media with vinasse for *Chlamydomonas reinhardii* for possible reduction in vinasse pollution. *Bioresour. Technol.* **1992**, *42*, 1–5. [CrossRef]
103. Marques, S.S.I.; Nascimento, I.A.; Almeida, P.F.; Chinalia, F.A. Growth of *Chlorella vulgaris* on sugarcane vinasse: The effect of anaerobic digestion pretreatment. *Appl. Biochem. Biotechnol.* **2013**, *171*, 1933–1943. [CrossRef]
104. Santos, R.R.; Araújo, O.Q.F.; Medeiros, J.L.; Chaloub, R.M. Cultivation of *Spirulina maxima* in medium supplemented with sugarcane vinasse. *Bioresour. Technol.* **2016**, *204*, 38–48. [CrossRef]
105. Candido, C.; Lombardi, A.T. The physiology of *Chlorella vulgaris* grown in conventional and biodigested treated vinasses. *Algal Res.* **2018**, *30*, 79–85. [CrossRef]
106. Soto, M.F.; Diaz, C.A.; Zapata, A.M.; Higuita, J.C. BOD and COD removal in vinasse from sugarcane alcoholic distillation by *Chlorella vulgaris*: Environmental evaluation. *Biochem. Eng. J.* **2021**, *176*, 108191. [CrossRef]
107. Pires, J.F.; Ferreira, G.M.R.; Reis, K.C.; Schwan, R.F.; Silva, C.F. Mixed yeasts inocula for simultaneous production of SCP and treatment of vinasse to reduce soil and fresh water pollution. *J. Environ. Manag.* **2016**, *182*, 455–463. [CrossRef] [PubMed]
108. Ricci, J.C.D.; Callieri, D.A.S.; Garro, O. Determination of the optimal conditions for the continuous culture of *Candida utilis* in sugarcane stillage. *Appl. Microbiol. Biotechnol.* **1987**, *27*, 100–104.
109. Santos, J.F.; Canettieri, E.V.; Souza, S.M.A.; Rodrigues, R.C.L.B.; Martínez, E.C. Treatment of sugarcane vinasse from cachaça production for the obtainment of *Candida utilis* CCT 3469 biomass. *Biochem. Eng. J.* **2019**, *148*, 131–137. [CrossRef]
110. Nunes, N.S.P.; Almeida, J.M.O.; Fonseca, G.G.; Carvalho, E.M. Clarification of sugarcane (*Saccharum offcinarum*) vinasse for microalgae cultivation. *Bioresour. Technol. Rep.* **2022**, *19*, 101125. [CrossRef]
111. Kahraman, S.S.; Gurdal, I.H. Effect of synthetic and natural culture media on laccase production by white rot fungi. *Bioresour. Technol.* **2002**, *82*, 215–217. [CrossRef] [PubMed]
112. Colin, V.L.; Cortes, A.A.J.; Aparicio, J.D.; Amoroso, M.J. Potential application of a bioemulsifier-producing actinobacterium for treatment of vinasse. *Chemosphere* **2016**, *144*, 842–847. [CrossRef]
113. Bastos, R.G.; Morais, D.V.; Volpi, M.P.C. Influence of solid moisture and bed height on cultivation of *Aspergillus niger* from sugarcane bagasse with vinasse. *Braz. J. Chem. Eng.* **2015**, *32*, 377–384. [CrossRef]
114. Menezes, E.G.T.; Alves, J.G.L.F.; Valeriano, C.; Guimarães, I.C. Physico-chemical and sensorial evaluation of sugarcane spirits produced using distillation residue. *Braz. Arch. Biol. Technol.* **2013**, *56*, 121–126. [CrossRef]
115. Yang, F.C.; Lin, I.H. Production of acid protease using thin stillage from a rice-spirit distillery by *Aspergillus niger*. *Enzyme Microb. Technol.* **1998**, *23*, 397–402. [CrossRef]
116. Hsieh, C.; Hsu, T.H.; Yang, F.C. Production of polysaccharides of *Ganoderma lucidum* (CCRC36021) by reusing thin stillage. *Process Biochem.* **2005**, *40*, 909–916. [CrossRef]
117. Liang, Y.; Zhao, X.; Strait, M.; Wen, Z. Use of dry-milling derived thin stillage for producing eicosapentaenoic acid (EPA) by the fungus *Pythium irregulare*. *Bioresour. Technol.* **2012**, *111*, 404–409. [CrossRef] [PubMed]
118. Yen, H.W.; Yang, Y.C.; Yu, Y.H. Using crude glycerol and thin stillage for the production of microbial lipids through the cultivation of *Rhodotorula glutinis*. *J. Biosci. Bioeng.* **2012**, *114*, 453–456. [CrossRef] [PubMed]
119. Wu, J.M.; Liu, R.H. Thin stillage supplementation greatly enhances bacterial cellulose production by *Gluconacetobacter xylinus*. *Carbohydr. Polym.* **2012**, *90*, 116–121. [CrossRef] [PubMed]
120. Wu, J.M.; Liu, R.H. Cost-effective production of bacterial cellulose in static cultures using distillery wastewater. *J. Biosci. Bioeng.* **2013**, *115*, 284–290. [CrossRef]
121. Khan, N.H.; Kang, T.S.; Grahame, D.A.S.; Haakensen, M.C.; Ratanapariyanuch, K.; Reaney, M.J.; Korber, D.R.; Tanaka, T. Isolation and characterization of novel 1;3-propanediol-producing *Lactobacillus panis* PM1 from bioethanol thin stillage. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 417–428. [CrossRef]
122. Wang, L.; Dong, X.; Jiang, H.; Li, G.; Zhang, M. Preparation of a novel carbon-based solid acid from cassava stillage residue and its use for the esterification of free fatty acids in waste cooking oil. *Bioresour. Technol.* **2014**, *158*, 392–395. [CrossRef] [PubMed]
123. Djukić-Vuković, A.P.; Mojović, L.V.; Semenčenko, V.V.; Radosavljević, M.M.; Pejin, J.D.; Kocić-Tanackov, S.D. Effective valorisation of distillery stillage by integrated production of lactic acid and high quality feed. *Food Res. Int.* **2015**, *73*, 75–80. [CrossRef]
124. Kim, S.M.; Li, S.; Pan, S.C.; Ding, Y.; Basu, R.; van Egmond, P.; Singh, V. A whole stillage sieving process to recover fiber for cellulosic ethanol production. *Ind. Crops Prod.* **2016**, *92*, 271–276. [CrossRef]
125. Ray, S.G.; Ghangrekar, M.M. Biodegradation kinetics of thin-stillage treatment by *Aspergillus awamori* and characterization of recovered chitosan. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1955–1965. [CrossRef]
126. Pereira, T.J. Study of the Use of Vinasse in the Preparation of the Yeast and in the Alcoholic Fermentation. Master's Thesis, University of Ribeirão Preto, Ribeirão Preto, Brazil, 2009. (In Portuguese)
127. Madaleno, L.L.; Barros, V.G.; Kesserling, M.A.; Teixeira, J.R.; Duda, R.M.; Oliveira, R.A. The recycling of biodigested vinasse in an upflow anaerobic sludge blanket reactor is a feasible approach for the conservation of freshwater in the biofuel ethanol industry. *J. Clean. Prod.* **2020**, *262*, 121196. [CrossRef]

128. Bialas, W.; Szymanowska, D.; Grajek, W. Fuel ethanol production from granular corn starch using *Saccharomyces cerevisiae* in a long term repeated SSF process with full stillage recycling. *Bioresour. Technol.* **2010**, *101*, 3126–3131. [CrossRef] [PubMed]
129. Gibbons, W.R.; Westby, C.A. Processing cereal grains; thin stillage; and cheese whey to fuel ethanol in a farm-scale plant. *Biomass* **1988**, *15*, 25–43. [CrossRef]
130. Wang, K.; Zhang, J.H.; Liu, P.; Cao, H.S.; Mao, Z.G. Reusing a mixture of anaerobic digestion effluent and thin stillage for cassava ethanol production. *J. Clean. Prod.* **2014**, *75*, 57–63. [CrossRef]
131. Zi, L.H.; Liu, C.G.; Xin, C.B.; Bai, F.W. Stillage backset and its impact on ethanol fermentation by the flocculating yeast. *Process Biochem.* **2013**, *48*, 753–758. [CrossRef]
132. Gurgel, M.N.A.; Correa, S.T.R.; Dourado Neto, D.; Paula Júnior, D.R. Technology for sugarcane agroindustry waste reuse as granulated organomineral fertilizer. *Eng. Agríc.* **2015**, *35*, 63–75. [CrossRef]
133. Nandy, T.; Shastry, S.; Kaul, S.N. Wastewater management in a cane molasses distillery involving bioresource recovery. *J. Environ. Manag.* **2002**, *65*, 25–38. [CrossRef] [PubMed]
134. Santos, J.D.; Silva, A.L.L.; Costa, J.L.; Scheidt, G.N.; Novak, A.C.; Sydney, E.B.; Soccol, C.R. Development of a vinasse nutritive solution for hydroponics. *J. Environ. Manag.* **2013**, *114*, 8–12. [CrossRef] [PubMed]
135. Akram, M.; Tan, C.K.; Garwood, R.; Thai, S.M. Vinasse—A potential biofuel—Cofiring with coal in a fluidised combustor. *Fuel* **2015**, *158*, 1006–1015. [CrossRef]
136. Cortez, L.A.B.; Pérez, L.E.B. Experiences on vinasse disposal: Part III: Combustion of vinasse–#6 fuel oil emulsions. *Braz. J. Chem. Eng.* **1997**, *14*. [CrossRef]
137. Crivelaro, S.H.R.; Mariano, A.P.; Furlan, L.T.; Gonçalves, R.A.; Seabra, P.N.; Angelis, D.F. Evaluation of the use of vinasse as a biostimulation agent for the biodegradation of oily sludge in soil. *Braz. Arch. Biol. Technol.* **2010**, *53*, 1217–1224. [CrossRef]
138. Mariano, A.P.; Crivelaro, S.H.R.; Angelis, D.F.; Bonotto, D.M. The use of vinasse as an amendment to ex-situ bioremediation of soil and groundwater contaminated with diesel oil. *Braz. Arch. Biol. Technol.* **2009**, *52*, 1043–1055. [CrossRef]
139. Tamashiro, J.R.; Kinoshita, A.; Silva, L.H.P.; Paiva, F.F.G.; Antunes, P.A.; Simões, R.D. Compressive resistance of concrete produced with recycled concrete aggregate and sugarcane vinasse waste-water. *Clean. Eng. Technol.* **2022**, *6*, 100362. [CrossRef]
140. Carpanez, T.G.; Moreira, V.R.; Assis, I.R.; Amaral, M.C.S. Sugarcane vinasse as organi-mineral fertilizers feedstock: Opportunities and environmental risks. *Sci. Total Environ.* **2022**, *832*, 154998. [CrossRef] [PubMed]
141. Madejón, E.; López, R.; Murillo, J.M.; Cabrera, F. Agricultural use of three (sugar-beet) vinasse composts: Effect on crops and chemical properties of a Cambisol soil in the Guadalquivir river valley (SW Spain). *Agr. Ecosyst. Environ.* **2001**, *84*, 55–65. [CrossRef]
142. Tejada, M.; Gonzalez, J.L. Effects on two beet vinasse forms on soil physical properties and soil loss. *Catena* **2006**, *68*, 41–50. [CrossRef]
143. Tejada, M.; Garcia, C.; Gonzalez, J.L.; Hernandez, M.T. Organic amendment based on fresh and composted beet vinasses: Influence on soil properties and wheat yield. *Soil Sci. Soc. Am. J.* **2006**, *70*, 900–908. [CrossRef]
144. Cruz, L.F.L.S.; Duarte, C.G.; Malheiros, T.F.; Pires, E.C. Technical, economic and environmental viability analysis of the current vinasse use: Ferti-irrigation, concentration and bio-digestion. *Braz. J. Environ. Sci. RBCIAMB* **2013**, *29*, 111–127. (In Portuguese)
145. Lameloise, M.L.; Gavach, M.; Bouix, M.; Fargues, C. Combining reverse osmosis and ion-exchange allows beet distillery condensates to be recycled as fermentable dilution water. *Desalination* **2015**, *363*, 75–81. [CrossRef]
146. Peiter, F.S.; Hankins, N.P.; Pires, E.C. Evaluation of concentration technologies in the design of biorefineries for the recovery or resources from vinasse. *Water Res.* **2019**, *157*, 483–497. [CrossRef]
147. Annachhatre, A.P. Anaerobic treatment of industrial wastewaters. *Resour. Conserv. Recy.* **1996**, *16*, 161–166. [CrossRef]
148. Gujer, W.; Zehnder, A.J.B. Conversion processes in anaerobic digestion. *Water Sci. Technol.* **1983**, *15*, 127–167. [CrossRef]
149. Khanal, S.K. *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications*, 1st ed.; Blackwell Publishing: New Delhi, India, 2008.
150. McCarty, P.L.; Smith, D.P. Anaerobic wastewater treatment. *Environ. Sci. Technol.* **1986**, *20*, 1200–1206. [CrossRef]
151. Mosey, F.E. Mathematical modeling of the anaerobic digestion process: Regulatory mechanisms for the formation of short-chain volatile acids from glucose. *Water Sci. Technol.* **1983**, *15*, 209–232. [CrossRef]
152. Stams, A.J.M. Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie Van Leeuwenhoek* **1994**, *66*, 271–294. [CrossRef] [PubMed]
153. Rajeshwari, K.V.; Balakrishnan, M.; Kansal, A.; Lata, K.; Kishore, V.V.N. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renew. Sustain. Energy Rev.* **2000**, *4*, 135–156. [CrossRef]
154. van Lier, J.B. High-rate anaerobic wastewater treatment: Diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. *Water Sci. Technol.* **2008**, *57*, 1137–1148. [CrossRef] [PubMed]
155. van Lier, J.B.; van der Zee, F.P.; Frijters, C.T.M.J.; Ersahin, M.E. Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 681–702. [CrossRef]
156. Deh Kiani, M.K.; Parsaee, M.; Ardebili, S.M.S.; Reyes, I.P.; Fuess, L.T.; Karimi, K. Different bioreactor configurations for biogas production from sugarcane vinasse: A comprehensive review. *Biomass Bioenergy* **2022**, *161*, 106446. [CrossRef]
157. Moletta, R. Winery and distillery wastewater treatment by anaerobic digestion. *Water Sci. Technol.* **2005**, *51*, 137–144. [CrossRef]
158. Bailey, J.E.; Ollis, D.F. *Biochemical Engineering Fundamentals*, 2nd ed.; McGraw-Hill: New York, NY, USA, 1986.

159. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [CrossRef]
160. Chernicharo, C.A.L. *Anaerobic reactors*, 1st ed.; IWA Publishing: London, UK, 2007.
161. Speece, R.E. *Anaerobic Biotechnology for Industrial Wastewaters*; Archae Press: Nashville, TN, USA, 1996.
162. Boncz, M.A.; Formagini, E.L.; Santos, L.S.; Marques, R.D.; Paulo, P.L. Application of urea dosing for alkalinity supply during anaerobic digestion of vinasse. *Water Sci. Technol.* **2012**, *66*, 2453–2460. [CrossRef] [PubMed]
163. Fuess, L.T.; Araújo, M.M., Jr.; Garcia, M.L.; Zaiat, M. Designing full-scale biodigestion plants for the treatment of vinasse in sugarcane biorefineries: How phase separation and alkalinization impact biogas and electricity production costs? *Chem. Eng. Res. Des.* **2017**, *119*, 209–220. [CrossRef]
164. Godoi, L.A.G.; Damianovic, M.H.R.; Foresti, E. Sulfidogenesis interference on methane production from carbohydrate-rich wastewater. *Water Sci. Technol.* **2015**, *72*, 1644–1652. [CrossRef] [PubMed]
165. Lens, P.N.L.; Visser, A.; Janssen, A.J.H.; Hulshoff Pol, L.W.; Lettinga, G. Biotechnological treatment of sulfate-rich wastewaters. *Crit. Rev. Environ. Sci. Technol.* **1998**, *28*, 41–88. [CrossRef]
166. Damianovic, M.H.R.Z.; Foresti, E. Dynamics of sulfidogenesis associated to methanogenesis in horizontal-flow anaerobic immobilized biomass reactor. *Process Biochem.* **2009**, *44*, 1050–1054. [CrossRef]
167. Anzola-Rojas, M.P.; Zaiat, M.; De Wever, H. Improvement of hydrogen production via ethanol-type fermentation in an anaerobic down-flow structured bed reactor. *Bioresour. Technol.* **2016**, *202*, 42–49. [CrossRef]
168. Saady, N.M.C. Homoacetogenesis during hydrogen production by mixed cultures dark fermentation. *Int. J. Hydrogen Energy* **2013**, *38*, 13172–13191. [CrossRef]
169. Matsumoto, M.; Nishimura, Y. Hydrogen production by fermentation using acetic acid and lactic acid. *J. Biosci. Bioeng.* **2007**, *103*, 236–241. [CrossRef]
170. Seeliger, S.; Janssen, P.H.; Schink, B. Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA. *FEMS Microbiol. Lett.* **2002**, *211*, 65–70. [CrossRef]
171. Thauer, R.K.; Jungermann, K.; Decker, K. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* **1977**, *41*, 100–180. [CrossRef]
172. Zhou, J.; Xing, J. Effect of electron donors on the performance of haloalkaliphilic sulfate-reducing bioreactors for flue gas treatment and microbial degradation patterns related to sulfate reduction of different electron donors. *Biochem. Eng. J.* **2015**, *96*, 14–22. [CrossRef]
173. Bertolino, S.M.; Melgaço, L.A.; Sá, R.G.; Leão, V.A. Comparing lactate and glycerol as single-electron donor for sulfate reduction in fluidized bed reactors. *Biodegradation* **2014**, *25*, 719–733. [CrossRef] [PubMed]
174. Costa, F.J.C.B.; Rocha, B.B.M.; Viana, C.E.; Toledo, A.C. Utilization of vinasse effluents from an anaerobic reactor. *Water Sci. Technol.* **1986**, *18*, 135–141. [CrossRef]
175. Russo, C.; Sant'Anna, G.L., Jr.; Pereira, S.E.C. An anaerobic filter applied to the treatment of distillery wastewaters. *Agric. Wastes* **1985**, *14*, 301–313. [CrossRef]
176. Barros, V.G.; Duda, R.M.; Oliveira, R.A. Biomethane production from vinasse in upflow anaerobic sludge blanket reactors inoculated with granular sludge. *Braz. J. Microbiol.* **2016**, *47*, 628–639. [CrossRef]
177. Mota, V.T.; Santos, F.S.; Amaral, M.C.S. Two-stage anaerobic membrane bioreactor for the treatment of sugarcane vinasse: Assessment on biological activity and filtration performance. *Bioresour. Technol.* **2013**, *146*, 494–503. [CrossRef]
178. Vuitik, G.A.; Fuess, L.T.; Del Nery, V.; Bañares-Alcántara, R.; Pires, E.C. Effects of recirculation in anaerobic baffled reactors. *J. Water Process Eng.* **2019**, *28*, 36–44. [CrossRef]
179. Rocha, M.H.; Elia Neto, A.; Salomon, K.R.; Lora, E.E.S.; Venturini, O.J.; del Olmo, A.O.; Rambla, M.A.O. Residues from biofuel production: Vinasse and glycerin. In *Biofuels*; Lora, E.E.S., Venturini, O.J., Eds.; Interciência: Rio de Janeiro, Brazil, 2012; Volume 2, pp. 691–809.
180. Acharya, B.K.; Mohana, S.; Madamwar, D. Anaerobic treatment of distillery spent wash—A study on upflow anaerobic fixed film bioreactor. *Bioresour. Technol.* **2008**, *99*, 4621–4626. [CrossRef]
181. España-Gamboa, E.; Mijangos-Cortés, J.; Hernández-Zárate, G.; Maldonado, J.A.D.; Alzate-Gaviria, L. Methane production by treating vinasses from hydrous ethanol using a modified UASB reactor. *Biotechnol. Biofuels.* **2012**, *5*, 82. [CrossRef]
182. Fernández, N.; Montalvo, S.; Borja, R.; Guerrero, L.; Sánchez, E.; Cortés, I.; Colmenarejo, M.F.; Travieso, L.; Raposo, F. Performance evaluation of an anaerobic fluidized bed reactor with natural zeolite as support material when treating high-strength distillery wastewater. *Renew. Energy* **2008**, *33*, 2458–2466. [CrossRef]
183. Kumar, G.S.; Gupta, S.K.; Singh, G. Biodegration of distillery spent wash in anaerobic hybrid reactor. *Water Res.* **2007**, *41*, 721–730. [CrossRef] [PubMed]
184. Salomon, K.R.; Lora, E.E.S. Estimate of the electric energy generating potential for different sources of biogas in Brazil. *Biomass Bioenergy* **2009**, *33*, 1101–1107. [CrossRef]
185. Janke, L.; Leite, A.F.; Batista, K.; Silva, W.; Nikolausz, M.; Nelles, M.; Stinner, W. Enhancing biogas production from vinasse in sugarcane biorefineries: Effects of urea and trace elements supplementation on process performance and stability. *Bioresour. Technol.* **2016**, *217*, 10–20. [CrossRef] [PubMed]
186. Bernal, A.P.; Santos, I.F.S.; Silva, A.P.M.; Barros, R.M.; Ribeiro, E.M. Vinasse biogas for energy generation in Brazil: An assessment of economic feasibility, energy potential and avoided CO_2 emissions. *J. Clean. Prod.* **2017**, *151*, 260–271. [CrossRef]

187. Fuess, L.T.; Garcia, M.L. Anaerobic digestion for enhanced bioenergy generation in ethanol biorefineries: Understanding the potentials of vinasse as a biofuel. In *Bioenergy Systems for the Future: Prospects for Biofuels and Biohydrogen*; Dalena, F., Basile, A., Rossi, C., Eds.; Woodhead Publishing: Cambridge, UK, 2017; pp. 149–183.
188. Nadaleti, W.C.; Lourenço, V.A.; Belli Filho, P.B.; Santos, G.B.; Przybyla, G. National potential production of methane and electrical energy from sugarcane vinasse in Brazil: A thermo-economic analysis. *J. Environ. Chem. Eng.* **2020**, *8*, 103422. [CrossRef]
189. Nogueira, C.E.C.; Souza, S.N.M.; Micuanski, V.C.; Azevedo, R.L. Exploring possibilities of energy insertion from vinasse biogas in the energy matrix of Paraná State, Brazil. *Renew. Sustain. Energy Rev.* **2015**, *48*, 300–305. [CrossRef]
190. Pereira, I.Z.; Santos, I.F.S.; Barros, R.M.; Castro e Silva, H.L.; Tiago Filho, G.L.; Moni e Silva, A.P. Vinasse biogas energy and economic analysis in the state of São Paulo, Brazil. *J. Clean. Prod.* **2020**, *260*, 121018. [CrossRef]
191. Rocha, M.H.; Lora, E.E.S.; Venturini, O.J.; Escobar, J.C.P.; Santos, J.J.C.S.; Moura, A.G. Use of the life cycle assessment (LCA) for comparison of the environmental performance of four alternatives for the treatment and disposal of bioethanol stillage. *Int. Sugar J.* **2010**, *112*, 611–622.
192. Moraes, B.S.; Palacios-Bereche, R.; Martins, G.; Nebra, S.A.; Fuess, L.T.; Silva, A.J.; Clementino, W.S.; Bajay, S.V.; Manduca, P.C.; Lamparelli, R.A.; et al. Biogas production: Technologies and applications. In *Biofuels and Biorefining: Current Technologies for Biomass Conversion*; Castro, F.I.G., Gutiérrez-Antonio, C., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; Volume 1, pp. 215–282.
193. Boddey, R.M.; Soares, L.H.B.; Alves, B.J.R.; Urquiaga, S. Bioethanol production in Brazil. In *Biofuels; Solar; and Wind as Renewable Energy Systems*; Pimentel, D., Ed.; Springer: Dordrecht, The Netherlands, 2008; pp. 321–356.
194. CGEE. *Sustainability of Sugarcane Bioenergy—Updated Edition*; CGEE: Brasília, Brazil, 2012. Available online: https://www.cgee.org.br/documents/10195/734063/Book_sustainabily_updated_3a_final06072012_9534.pdf/059bf982-ec47-4ce8-9ac1-dc53f093d4cf?version=1.4 (accessed on 27 September 2022).
195. Khan, A.S.; Fox, R.W. Net energy analyses of ethanol production from sugarcane in Northeast Brazil. *Biomass* **1982**, *2*, 213–221. [CrossRef]
196. Macedo, I.C. The sugar cane agro-industry—Its contribution to reducing CO_2 emissions in Brazil. *Biomass Bioenergy* **1992**, *3*, 77–80. [CrossRef]
197. Macedo, I.C. Greenhouse gas emissions and energy balances in bio-ethanol production and utilization in Brazil (1996). *Biomass Bioenergy* **1998**, *14*, 77–81. [CrossRef]
198. Macedo, I.C.; Seabra, J.E.A.; Silva, J.E.A.R. Green house gases emissions in the production and use of ethanol from sugarcane in Brazil: The 2005/2006 averages and a prediction for 2020. *Biomass Bioenergy* **2008**, *32*, 582–595. [CrossRef]
199. Oliveira, M.E.D.; Vaughan, B.E.; Rykiel, E.J., Jr. Ethanol as fuel: Energy; carbon dioxide balances; and ecological footprint. *Bioscience* **2005**, *55*, 593–602. [CrossRef]
200. Turdera, M.V. Energy balance; forecasting of bioelectricity generation and greenhouse gas emission balance in the ethanol production at sugarcane mills in the state of Mato Grosso do Sul. *Renew. Sustain. Energy Rev.* **2013**, *19*, 582–588. [CrossRef]
201. EPE. *2021 Brazilian Energy Balance—Year 2020*; Final Report; EPE: Rio de Janeiro, Brazil, 2021. Available online: https://www.epe.gov.br/pt/publicacoes-dados-abertos/publicacoes/balanco-energetico-nacional-2021 (accessed on 27 September 2022).
202. Ke, S.; Shi, Z.; Fang, H.H.P. Applications of two-phase anaerobic degradation in industrial wastewater treatment. *Int. J. Environ. Pollut.* **2005**, *23*, 65–80. [CrossRef]
203. Hallenbeck, P.C. Fermentative hydrogen production: Principles, progress, and prognosis. *Int. J. Hydrogen Energy* **2009**, *34*, 7379–7389. [CrossRef]
204. Liu, Z.; Zhang, C.; Lu, Y.; Wu, X.; Wang, L.; Wang, L.; Han, B.; Xing, X.H. States and challenges for high-value biohythane production from waste biomass by dark fermentation technology. *Bioresour. Technol.* **2013**, *135*, 292–303. [CrossRef]
205. Sarma, S.J.; Pachapur, V.; Kaur Brar, S.; Le Bihan, Y.; Buelna, G. Hydrogen biorefinery: Potential utilization of the liquid waste from fermentative hydrogen production. *Renew. Sustain. Energy Rev.* **2015**, *50*, 942–951. [CrossRef]
206. Yu, B.; Shan, A.; Zhang, D.; Lou, Z.; Yuan, H.; Huang, X.; Zhu, N.; Hu, X. Dosing time of ferric chloride to disinhibit the excessive volatile fatty acids in sludge thermophilic anaerobic digestion system. *Bioresour. Technol.* **2015**, *189*, 154–161. [CrossRef]
207. Holl, E.; Steinbrenner, J.; Merkle, W.; Krümpel, J.; Lansing, S.; Baier, U.; Oechsner, H.; Lemmer, A. Two-stage anaerobic digestion: State of technology and perspective roles in future energy systems. *Bioresour. Technol.* **2022**, *360*, 127633. [CrossRef]
208. Albanez, R.; Lovato, G.; Zaiat, M.; Ratusznei, S.M.; Rodrigues, J.A.D. Optimization, metabolic pathways modeling and scale-up estimative of an AnSBBR applied to biohydrogen production by co-digestion of vinasse and molasses. *Int. J. Hydrogen Energy* **2016**, *41*, 20473–20484. [CrossRef]
209. Ferraz, A.D.N., Jr.; Wenzel, J.; Etchebehere, C.; Zaiat, M. Effect of organic loading rate on hydrogen production from sugarcane vinasse in thermophilic acidogenic packed bed reactors. *Int. J. Hydrogen Energy* **2014**, *39*, 16852–16862.
210. Ferraz, A.D.N., Jr.; Etchebehere, C.; Zaiat, M. High organic loading rate on thermophilic hydrogen production and metagenomic study at an anaerobic packed-bed reactor treating a residual liquid stream of a Brazilian biorefinery. *Bioresour. Technol.* **2015**, *186*, 81–88. [CrossRef] [PubMed]
211. Ferraz, A.D.N., Jr.; Etchebehere, C.; Zaiat, M. Mesophilic hydrogen production in acidogenic packed-bed reactors (APBR) using raw sugarcane vinasse as substrate: Influence of support materials. *Anaerobe* **2015**, *34*, 94–105.
212. Lazaro, C.Z.; Perna, V.; Etchebehere, C.; Varesche, M.B.A. Sugarcane vinasse as substrate for fermentative hydrogen production: The effects of temperature and substrate concentration. *Int. J. Hydrogen Energy* **2014**, *39*, 6407–6418. [CrossRef]

213. Lazaro, C.Z.; Varesche, M.B.A.; Silva, E.L. Sequential fermentative and phototrophic system for hydrogen production: An approach for Brazilian alcohol distillery wastewater. *Int. J. Hydrogen Energy* **2015**, *40*, 9642–9655. [CrossRef]
214. Bernal, A.P.; Menezes, C.A.; Silva, E.L. A new side-looking at the dark fermentation of sugarcane vinasse: Improving the carboxylates production in mesophilic EGSB by selection of the hydraulic retention time and substrate concentration. *Int. J. Hydrogen Energy* **2021**, *46*, 12758–12770. [CrossRef]
215. Niz, M.Y.K.; Etchelet, I.; Fuentes, L.; Etchebehere, C.; Zaiat, M. Extreme thermophilic condition: An alternative for long-term biohydrogen production from sugarcane vinasse. *Int. J. Hydrogen Energy* **2019**, *44*, 22876–22887. [CrossRef]
216. Ramos, L.R.; Silva, E.L. Continuous hydrogen production from agricultural wastewaters at thermophilic and hyperthermophilic temperatures. *Appl. Biochem. Biotechnol.* **2017**, *182*, 846–869. [CrossRef]
217. Ramos, L.R.; Silva, E.L. Thermophilic hydrogen and methane production from sugarcane stillage in two-stage anaerobic fluidized bed reactors. *Int. J. Hydrogen Energy* **2020**, *45*, 5239–5251. [CrossRef]
218. Rego, G.C.; Ferreira, T.B.; Ramos, L.R.; Menezes, C.A.; Soares, L.A.; Sakamoto, I.K.; Varesche, M.B.A.; Silva, E.L. Bioconversion of pretreated sugarcane vinasse into hydrogen: New perspectives to solve one of the greatest issues of the sugarcane biorefinery. *Biomass Conv. Bioref.* **2022**, *12*, 5527–5541. [CrossRef]
219. Reis, C.M.; Carosia, M.F.; Sakamoto, I.K.; Varesche, M.B.A.; Silva, E.L. Evaluation of hydrogen and methane production from sugarcane vinasse in an anaerobic fluidized bed reactor. *Int. J. Hydrogen Energy* **2015**, *40*, 8498–8509. [CrossRef]
220. Santos, S.C.; Rosa, P.R.F.; Sakamoto, I.K.; Varesche, M.B.A.; Silva, E.L. Continuous thermophilic hydrogen production and microbial community analysis from anaerobic digestion of diluted sugar cane stillage. *Int. J. Hydrogen Energy* **2014**, *39*, 9000–9011. [CrossRef]
221. Santos, S.C.; Rosa, P.R.F.; Sakamoto, I.K.; Varesche, M.B.A.; Silva, E.L. Hydrogen production from diluted and raw sugarcane vinasse under thermophilic anaerobic conditions. *Int. J. Hydrogen Energy* **2014**, *39*, 9599–9610. [CrossRef]
222. Santos, S.C.; Rosa, P.R.F.; Sakamoto, I.K.; Varesche, M.B.A.; Silva, E.L. Organic loading rate impact on biohydrogen production and microbial communities at anaerobic fluidized thermophilic bed reactors treating sugarcane stillage. *Bioresour. Technol.* **2014**, *159*, 55–63. [CrossRef] [PubMed]
223. Heywood, J.B. *Internal Combustion Engine Fundamentals*, 1st ed.; McGraw-Hill: New York, NY, USA, 1988.
224. Pawar, S.S.; van Niel, E.W.J. Thermophilic biohydrogen production: How far are we? *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7999–8009. [CrossRef] [PubMed]
225. Karthic, P.; Shiny, J. Comparison and limitations of biohydrogen production processes. *Res. J. Biotechnol.* **2012**, *7*, 59–71.
226. Bartels, J.R.; Pate, M.B.; Olson, N.K. An economic survey of hydrogen production from conventional and alternative energy sources. *Int. J. Hydrogen Energy* **2010**, *35*, 8371–8384. [CrossRef]
227. Chandra, R.; Mohan, S.V. Enhanced bio-hydrogenesis by co-culturing photosynthetic bacteria with acidogenic process: Augmented dark-photo fermentative hybrid system to regulate volatile fatty acid inhibition. *Int. J. Hydrogen Energy* **2014**, *39*, 7604–7615. [CrossRef]
228. Fuess, L.T.; Ferraz, A.D.N., Jr.; Machado, C.B.; Zaiat, M. Temporal dynamics and metabolic correlation between lactate-producing and hydrogen-producing bacteria in sugarcane vinasse dark fermentation: The key role of lactate. *Bioresour. Technol.* **2018**, *247*, 426–433. [CrossRef]
229. Cavinato, C.; Giuliano, A.; Bolzonella, D.; Pavan, P.; Cecchi, F. Bio-hythane production from food waste by dark fermentation coupled with anaerobic digestion process: A long-term pilot scale experience. *Int. J. Hydrogen Energy* **2012**, *37*, 11549–11555. [CrossRef]
230. Noori, M.T.; Min, B. Fundamentals and recent progress in bioelectrochemical system-assisted biohythane production. *Bioresour. Technol.* **2022**, *361*, 127641. [CrossRef] [PubMed]
231. Çeper, B.A. Use of hydrogen-methane blends in internal combustion engines. In *Hydrogen Energy—Challenges and Perspectives*; Minic, D., Ed.; InTech: Rijeka, Croatia, 2012; pp. 175–200.
232. Ma, F.; Naeve, N.; Wang, M.; Jiang, L.; Chen, R.; Zhao, S. Hydrogen-enriched compressed natural gas as a fuel for engines. In *Natural Gas*; Potocnik, M., Ed.; InTech: Rijeka, Croatia, 2012; pp. 307–332.
233. Luo, G.; Angelidaki, I. Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture. *Biotechnol. Bioeng.* **2012**, *109*, 2729–2736. [CrossRef] [PubMed]
234. Luo, G.; Johansson, S.; Boe, K.; Xie, L.; Zhou, Q.; Angelidaki, I. Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor. *Biotechnol. Bioeng.* **2012**, *109*, 1088–1094. [CrossRef] [PubMed]
235. Zhao, J.; Li, Y.; Dong, R. Recent progress towards in-situ biogas upgrading Technologies. *Sci. Total Environ.* **2021**, *800*, 149667. [CrossRef]
236. Show, K.Y.; Lee, D.J.; Tay, J.H.; Lin, C.Y.; Chang, J.S. Biohydrogen production: Current perspectives and the way forward. *Int. J. Hydrogen Energy* **2012**, *37*, 15616–15631. [CrossRef]
237. Silva, A.J.; Pozzi, E.; Foresti, E.; Zaiat, M. The influence of the buffering capacity on the production of organic acids and alcohols from wastewater in anaerobic reactor. *Appl. Biochem. Biotechnol.* **2014**, *175*, 2258–2265. [CrossRef]
238. Eng, F.; Fuess, L.T.; Bovio-Winkler, P.; Etchebehere, C.; Sakamoto, I.K.; Zaiat, M. Optimization of volatile fatty acid production by sugarcane vinasse dark fermentation using a response surface methodology. Links between performance and microbial community composition. *Sustain. Energy Technol. Assess.* **2022**, *53*, 102764.

239. Antonopoulou, G.; Gavala, H.N.; Skiadas, I.V.; Lyberatos, G. Influence of pH on fermentative hydrogen production from sweet sorghum extract. *Int. J. Hydrogen Energy* **2010**, *35*, 1921–1928. [CrossRef]
240. Jankowska, E.; Chwiałkowska, J.; Stodolny, M.; Oleskowicz-Popiel, P. Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. *Bioresour. Technol.* **2015**, *190*, 274–280. [CrossRef]
241. Xie, L.; Liu, H.; Chen, Y.G.; Zhou, Q. pH-adjustment strategy for volatile fatty acid production from high-strength wastewater for biological nutrient removal. *Water Sci. Technol.* **2014**, *69*, 2043–2051. [CrossRef] [PubMed]
242. Sjöblom, M.; Matsakas, L.; Christakopoulos, P.; Rova, U. Production of butyric acid by *Clostridium tyrobutyricum* (ATCC25755) using sweet sorghum stalks and beet molasses. *Ind. Crops Prod.* **2015**, *74*, 535–544. [CrossRef]
243. Regestein, L.; Doerr, E.W.; Staaden, A.; Rehmann, L. Impact of butyric acid on butanol formation by *Clostridium pasteurianum*. *Bioresour. Technol.* **2015**, *196*, 153–159. [CrossRef]
244. Pereira, L.G.; Dias, M.O.S.; Mariano, A.P.; Maciel Filho, R.; Bonomi, A. Economic and environmental assessment of n-butanol production in an integrated first and second generation sugarcane biorefinery: Fermentative versus catalytic routes. *Appl. Energy* **2015**, *160*, 120–131. [CrossRef]
245. Vieira, C.F.S.; Sia, A.D.; Maugeri Filho, F.; Maciel Filho, R.; Mariano, A.P. Isopropanol-butanol-ethanol production by cell-immobilized vacuum fermentation. *Bioresour. Technol.* **2022**, *344*, 126313. [CrossRef] [PubMed]
246. Zetty-Arenas, A.M.; Tovar, L.P.; Alves, R.F.; Mariano, A.P.; van Gulik, W.; Maciel Filho, R.; Freitas, S. Co-fermentation of sugarcane bagasse hydrolysate and molasses *Clostridium saccharoperbutylacetonicum*: Effect on sugar consumption and butanol production. *Ind. Crops Prod.* **2021**, *167*, 113512. [CrossRef]
247. Jang, Y.S.; Im, J.A.; Choi, S.Y.; Lee, J.I.; Lee, S.Y. Metabolic engineering of *Clostridium acetobutylicum* for butyric acid production with high butyric acid selectivity. *Metab. Eng.* **2014**, *23*, 165–174. [CrossRef]
248. Sydney, E.B.; Larroche, C.; Novak, A.C.; Nouaille, R.; Sarma, S.J.; Kaur Brar, S.; Letti, L.A.J.; Soccol, V.T.; Soccol, C.R. Economic process to produce biohydrogen and volatile fatty acids by a mixed culture using vinasse from sugarcane ethanol industry as nutrient source. *Bioresour. Technol.* **2014**, *159*, 380–386. [CrossRef]
249. Fuess, L.T.; Santos, G.M.; Delforno, T.P.; Moraes, B.S.; Silva, A.J. Biochemical butyrate production via dark fermentation as an energetically efficient alternative management approach for vinasse in sugarcane biorefineries. *Renew. Energy* **2020**, *158*, 3–12. [CrossRef]
250. Możejko-Ciesielska, J.; Kiewisz, R. Bacterial polyhydroxyalkanoates: Still fabulous? *Microbiol. Res.* **2016**, *192*, 271–282. [CrossRef]
251. Amulya, K.; Reddy, M.V.; Mohan, S.V. Acidogenic spent wash valorization through polyhydroxyalkanoate (PHA) synthesis coupled with fermentative biohydrogen production. *Bioresour. Technol.* **2014**, *158*, 336–342. [CrossRef] [PubMed]
252. Marang, L.; Jiang, Y.; van Loosdrecht, M.C.M.; Kleerebezem, R. Butyrate as preferred substrate for polyhydroxybutyrate production. *Bioresour. Technol.* **2013**, *142*, 232–239. [CrossRef] [PubMed]
253. Reddy, M.V.; Nikhil, G.N.; Mohan, S.V.; Swamy, Y.V.; Sarma, P.N. *Pseudomonas otitidis* as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. *Bioresour. Technol.* **2012**, *123*, 471–479. [CrossRef]
254. Liu, B.; Wen, Q.; Huang, L.; Chen, Z.; Lin, X.; Liu, S. Insights into integration of polyhydroxyalkanoates (PHAs) production into wastewater treatment: Comparison of different electron acceptors on system function and PHA-producer enrichment. *Chem. Eng. J.* **2023**, *451*, 138631. [CrossRef]
255. Nonato, R.V.; Mantelatto, P.E.; Rossell, C.E.V. Integrated production of biodegradable plastic; sugar and ethanol. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 1–5.
256. Radivojevic, J.; Skaro, S.; Senerovic, L.; Vasiljevic, B.; Guzik, M.; Kenny, S.T.; Maslak, V.; Nikodinovic-Runic, J.; O'Connor, K.E. Polyhydroxyalkanoate-based 3-hydroxyoctanoic acid and its derivatives as a platform of bioactive compounds. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 161–172. [CrossRef] [PubMed]
257. García-Depraect, O.; Lebrero, R.; Rodriguez-Vega, S.; Bordel, S.; Santos-Beneit, F.; Martínez-Mendoza, L.J.; Börner, R.A.; Börner, T.; Muñoz, R. Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: Kinetics, carbon fate and particle size effect. *Bioresour. Technol.* **2022**, *344*, 126265. [CrossRef] [PubMed]
258. García-Depraect, O.; Lebrero, R.; Rodriguez-Vega, S.; Börner, R.A.; Börner, T.; Muñoz, R. Production of volatile fatty acids (VFAs) from five commercial bioplastics via acidogenic fermentation. *Bioresour. Technol.* **2022**, *360*, 127655. [CrossRef] [PubMed]
259. Mohan, S.V.; Reddy, M.V.; Subhash, G.V.; Sarma, P.N. Fermentative effluents from hydrogen producing bioreactor as substrate for poly(β-OH) butyrate production with simultaneous treatment: An integrated approach. *Bioresour. Technol.* **2010**, *101*, 9382–9386. [CrossRef]
260. Albuquerque, M.G.E.; Eiroa, M.; Torres, C.; Nunes, B.R.; Reis, M.A.M. Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. *J. Biotechnol.* **2007**, *130*, 411–421. [CrossRef]
261. Oliveira, G.H.D.; Niz, M.Y.K.; Zaiat, M.; Rodrigues, J.A.D. Effects of organic loading rate on polyhydroxyalkanoate production from sugarcane stillage by mixed microbial cultures. *Appl. Biochem. Biotechnol.* **2019**, *189*, 1039–1055. [CrossRef] [PubMed]
262. Silverio, M.S.; Piccoli, R.A.M.; Reis, J.L.M.S.; Gomez, J.G.C.; Baptista, A.S. Techno-economic feasibility of P(3-hydroxybutyrate) bioprocess with concentrated sugarcane vinasse as carbon and minerals source: An experimental and in silico approach. *Biomass Conv. Bioref.* **2022**. [CrossRef]

263. Bekatorou, A.; Dima, A.; Tsafrakidou, P.; Boura, K.; Lappa, K.; Kandylis, P.; Pissaridi, K.; Kanellaki, M.; Koutinas, A.A. Downstream extraction process development for recovery of organic acids from a fermentation broth. *Bioresour. Technol.* **2016**, *220*, 34–37. [CrossRef] [PubMed]
264. Reis, M.A.M.; Gonçalves, L.M.D.; Carrondo, M.J.T. Sulphate removal in acidogenic phase anaerobic digestion. *Environ. Technol.* **1988**, *9*, 775–784. [CrossRef]
265. Reis, M.A.M.; Lemos, P.C.; Martins, M.J.; Costa, P.C.; Gonçalves, L.M.D.; Carrondo, M.J.T. Influence of sulfates and operational parameters on volatile fatty acids concentration profile in acidogenic phase. *Bioprocess Eng.* **1991**, *6*, 145–151. [CrossRef]
266. Piffer, M.A.; Oliveira, C.A.; Bovio-Winkler, P.; Eng, F.; Etchebehere, C.; Zaiat, M.; Nascimento, C.A.O.; Fuess, L.T. Sulfate- and pH-driven metabolic flexibility in sugarcane vinasse dark fermentation stimulates biohydrogen evolution, sulfidogenesis or homoacetogenesis. *Int. J. Hydrogen Energy* **2022**, *47*, 31202–31222. [CrossRef]
267. Junqueira, T.L.; Moraes, B.; Gouveia, V.L.R.; Chagas, M.F.; Morais, E.R.; Watanabe, M.D.B.; Zaiat, M.; Bonomi, A. Use of VSB to plan research programs and public policies. In *Virtual Biorefinery: An Optimization Strategy for Renewable Carbon Valorization*; Bonomi, A., Cavalett, O., Cunha, M.P., Lima, M.A.P., Eds.; Springer: London, UK, 2016; pp. 257–282.
268. Souza, S.N.M.; Borsoi, A.; Santos, R.F.; Secco, D.; Frigo, E.P.; Silva, M.J. Production potential of biogas in sugar and ethanol plants for use in urban buses in Brazil. *J. Food Agric. Environ.* **2012**, *10*, 908–910.
269. Wilkie, A.C. Biomethane from biomass, biowaste and biofuels. In *Bioenergy*; Wall, J.D., Harwood, C.S., Demain, A., Eds.; ASM Press: Washington, DC, USA, 2008; pp. 195–205.
270. Fuess, L.T.; Zaiat, M.; Nascimento, C.A.O. Can biogas-producing sugarcane biorefineries techno-economically outperform conventional ethanol production? Deciphering the way towards maximum profitability. *Energy Convers. Manag.* **2022**, *254*, 115206. [CrossRef]
271. ANP. Resolution ANP n. 685, 29 June 2017. Brasília, DF, Brazil. 2017. Available online: https://atosoficiais.com.br/anp/resolucao-n-685-2017-estabelece-as-regras-para-aprovacao-do-controle-da-qualidade-e-a-especificacao-do-biometano-oriundo-de-aterros-sanitarios-e-de-estacoes-de-tratamento-de-esgoto-destinado-ao-uso-veicular-e-as-instalacoes-residenciais-industriais-e-comerciais-a-ser-comercializado-em-todo-o-territorio-nacional?origin=instituicao&q=685/2017 (accessed on 27 September 2022).
272. Barrera, E.L.; Rosa, E.; Spanjers, H.; Romero, O.; De Meester, S.; Dewulf, J. A comparative assessment of anaerobic digestion power plants as alternative to lagoons for vinasse treatment: Life cycle assessment and exergy analysis. *J. Clean. Prod.* **2016**, *113*, 459–471. [CrossRef]
273. Muñoz, R.; Meier, L.; Diaz, I.; Jeison, D. A critical review on the state-of-the-art of physical/chemical and biological technologies for an integral biogas upgrading. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 727–759. [CrossRef]
274. Leme, R.M.; Seabra, J.E.A. Technical-economic assessment of different biogas upgrading routes from vinasse anaerobic digestion in the Brazilian bioethanol industry. *Energy* **2017**, *119*, 754–766. [CrossRef]
275. Allegue, L.B.; Hinge, J. *Biogas Upgrading—Evaluation of Methods for H_2S Removal*; Danish Technological Institute: Taastrup, Denmark, 2014. Available online: https://www.teknologisk.dk/_/media/60599_Biogas%20upgrading.%20Evaluation%20of%20methods%20for%20H2S%20removal.pdf (accessed on 27 September 2022).
276. Lebrero, R.; Toledo-Cervantes, A.; Muñoz, R.; Del Nery, V.; Foresti, E. Biogas upgrading from vinasse digesters: A comparison between an anoxic biotrickling filter and an algal-bacterial photobioreactor. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 2488–2495. [CrossRef]
277. Dias, M.F.; Colturato, L.F.; Oliveira, J.P.; Leite, L.R.; Oliveira, G.; Chernicharo, C.A.; Araújo, J.C. Metagenomic analysis of a desulphurisation system used to treat biogas from vinasse methanisation. *Bioresour. Technol.* **2016**, *205*, 58–66. [CrossRef]
278. Jespersen, N.D. *Barron's AP Chemistry*, 4th ed.; Barron's Education Series, Inc.: New York, NY, USA, 2007.
279. US Geological Survey. *Mineral Commodity Summaries 2022*; US Geological Survey: Reston, VA, USA, 2022. Available online: https://pubs.usgs.gov/periodicals/mcs2022/mcs2022.pdf (accessed on 19 September 2022).
280. EPA. Catalog of CHP Technologies. US Environmental Protection Agency, Combined Heat and Power Partnership: Washington, DC, USA, 2017. Available online: https://www.epa.gov/sites/production/files/2015-07/documents/catalog_of_chp_technologies.pdf (accessed on 27 September 2022).
281. Fuess, L.T.; Cruz, R.B.C.M.; Zaiat, M.; Nascimento, C.A.O. Diversifying the portfolio of sugarcane biorefineries: Anaerobic digestion as the core process for enhanced resource recovery. *Renew. Sustain. Energy Rev.* **2021**, *147*, 111246. [CrossRef]
282. Moreira, A.; Moraes, L.A.C.; Aquino, G.S.; Heinrichs, R. Biofuel production from sugarcane: Various routes of harvesting energy from the crop. In *Sugarcane Biofuels: Status, Potential, and Prospects of the Sweet Crop to Fuel the World*; Khan, M.T., Khan, I.A., Eds.; Springer: Cham, Switzerland, 2019; pp. 21–38.
283. UN. *Transforming Our World: The 2030 Agenda for Sustainable Development*; A/RES/70/1; United Nations: New York, NY, USA, 2020. Available online: https://sustainabledevelopment.un.org/content/documents/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf (accessed on 27 September 2022).
284. Ge, X.; Xu, F.; Li, Y. Solid-state anaerobic digestion of lignocellulosic biomass: Recent progress and perspectives. *Bioresour. Technol.* **2016**, *205*, 239–249. [CrossRef] [PubMed]
285. Yang, L.; Xu, F.; Ge, X.; Li, Y. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renew. Sustain. Energy Rev.* **2015**, *44*, 824–834. [CrossRef]

286. Adarme, O.F.H.; Baêta, B.E.L.; Gabriel Filho, J.B.; Gurgel, L.V.A.; Aquino, S.F. Use of anaerobic co-digestion as an alternative to add value to sugarcane biorefinery wastes. *Bioresour. Technol.* **2019**, *287*, 121443. [CrossRef]
287. Barros, V.G.; Duda, R.M.; Vantini, J.S.; Omori, W.P.; Ferro, M.I.T.; Oliveira, R.A. Improved methane production from sugarcane vinasse with filter cake in thermophilic UASB reactors, with predominance of *Methanothermobacter* and *Methanosarcina archaea* and *Thermotogae bacteria*. *Bioresour. Technol.* **2017**, *244*, 371–381. [CrossRef]
288. González, L.M.L.; Reyes, I.P.; Romero, O.R. Anaerobic co-digestion of sugarcane press mud with vinasse on methane yield. *Waste Manag.* **2017**, *68*, 139–145. [CrossRef]
289. Volpi, M.P.C.; Fuess, L.T.; Moraes, B.S. Anaerobic co-digestion of residues in 1G2G sugarcane biorefineries for enhanced electricity and biomethane production. *Bioresour. Technol.* **2021**, *330*, 124999. [CrossRef]
290. Anupama; Ravindra, P. Value-added food: Single cell protein. *Biotechnol. Adv.* **2000**, *18*, 459–479. [CrossRef] [PubMed]
291. Jain, A.; Jayaraman, S.; Singh, G.; Srinivasan, M.P. Single step peroxidase extraction and oxidation of highly concentrated ethanol and phenol aqueous solutions using supercritical carbon dioxide. *J. Supercritic. Fluids* **2016**, *116*, 209–214. [CrossRef]
292. McClements, D.J.; Gumus, C.E. Natural emulsifiers—Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv. Colloid. Interface Sci.* **2016**, *234*, 3–26. [CrossRef] [PubMed]
293. Ribas, M.M.F. Vinasse Treatment in Anaerobic Sequencing Batch Reactor with Immobilized Biomass under Thermophilic and Mesophilic Conditions. Ph.D. Thesis, University of São Paulo, São Carlos, Brazil, 2006. (In Portuguese)
294. Heijnen, J.J.; Mulder, A.; Enger, W.; Hoeks, F. Review on the application of anaerobic fluidized bed reactors in waste-water treatment. *Chem. Eng. J.* **1989**, *41*, B37–B50. [CrossRef]
295. CONAB. *Thermoelectric Generation from Sugarcane Bagasse Burning in Brazil: Performance Assessment of the 2009/2010 Harvest*; CONAB: Brasília, Brazil, 2011. Available online: https://www.gov.br/agricultura/pt-br/assuntos/sustentabilidade/agroenergia/arquivos-termoeletrica-com-a-queima-do-bagaco-de-cana-de-acucar/termoeletrica-com-a-queima-do-bagaco-de-cana-de-acucar-no-brasil-safra-2009-2010.pdf (accessed on 23 September 2022). (In Portuguese)