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Effect of Wet, Hemi-Solid, and Solid-State Conditions and Substrate to Inoculum Ratio on Methane Production from Sugarcane Bagasse

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Abstract: Sugarcane bagasse (SCB) constitutes up to 28% of the weight of crushed sugarcane, with significant potential for bioenergy production. Solid-state anaerobic digestion with total solids (TSs) over 15% is an interesting technology that can be used to treat agricultural wastes such as SCB, resulting in smaller reactor sizes and lower water consumption. This study investigates methane production from SCB under wet (10% TS), hemi-solid (15% TS), and solid-state (20% TS) anaerobic digestion with substrate-to-inoculum ratios (SIR) of 1, 2, 3, and 4. Batch experiments were conducted under mesophilic conditions (37 °C) to evaluate methane yields, volumetric methane productivity, and kinetic parameters. Results revealed that the highest methane yields—125, 115, and 106 L CH$_4$ kg VS^{-1}—were achieved for wet, hemi-solid, and solid-state digestion, respectively. Despite similar methane yields across TS conditions, volumetric methane productivities increased by 118% and 128% from hemi-slid and solid-state digestion, demonstrating their potential for scaling up in commercial biogas plants. The first-order kinetic model best-predicted methane production (R^2 > 0.984), with hydrolysis identified as the limiting step (K$_{hyd}$ ≤ 0.05 d^{-1}). These findings highlight the advantages of solid-state anaerobic digestion for lignocellulosic feedstocks like SCB, contributing to bioenergy sustainability and the circular economy.

Keywords: biogas; circular economy; dry digestion; sustainability; waste to energy

1. Introduction

The demand for cleaner and cheaper sources of energy to replace fossil fuels is increasing worldwide. The renewable energy obtained by the bioconversion of the carbohydrate content from lignocellulosic biomass into biofuels is an attractive option to meet part of the global energy demand and minimize associated environmental aspects of energy production and solid waste management [1]. Sugarcane is a widely cultivated crop used to produce sugar, livestock feed, and energy such as first-generation bioethanol. According to the International Sugar Organization [2], about 64 million tons per year of sugar is internationally traded, and Brazil, India, China, Thailand, the US, and Australia are among the top sugarcane producers.

Despite being considered one of the most efficient substrates for biofuel production, processing sugarcane causes significant environmental impacts, especially related to the storage and management of the surplus bagasse. About 25–28% of each ton of crushed sugarcane is generated as bagasse (SCB) and 65–75% of it is burned to produce low-pressure

steam and electricity in the sugar mills [3], resulting in 25–35% of the remaining bagasse available for second generation bioenergy production.

Anaerobic digestion (AD) is a sustainable and decentralized alternative to treat a wide range of biowastes such as animal manure, wastewater sludge, food waste, and crop residues. The two main by-products of AD are a green source of energy in the form of biogas, mainly composed of carbon dioxide (CO_2) and methane (CH_4), and a digestate that can be potentially used as a biofertilizer to improve soil fertility, which also contributes to the circular economy of nutrients [4]. AD is a multistage biological process conventionally divided into hydrolysis, acidogenesis, acetogenesis, and methanogenesis. According to the total solids (TS) content, the AD process can be classified as wet (W-AD) with TS < 10%, hemi-solid (HS-AD) with TS between 10 and 15%, and solid-state (SS-AD) with TS > 15%, the later also known as dry-digestion [5].

The moisture content of the feedstock and inoculum source are key factors that influence mass transfer between the gas–liquid–solid phases and the rate of substrate degradation during AD [6]. W-AD is widely applied to biomasses limited to high moisture content, such as animal manure and agro-industrial wastewater. However, it requires significant energy for piping, mixing, and heating and generates a substantial volume of liquid digestate to be managed. Nonetheless, SS-AD is suitable for agricultural wastes with low moisture and high lignocellulose content. It has some advantages over W-AD, such as higher volumetric methane productivity, lower consumption of water, smaller reactor sizes, lower energy requirements for heating and mixing, minimal material handling, positive energy balances, and the easiest management of end products [7]. Therefore, the adequate digestion of agricultural wastes like SCB, in addition to generating benefits such as optimized bioenergy production and the generation of a stabilized biofertilizer, can contribute to the circular economy indicators of agribusiness [8,9].

However, one of the main disadvantages of SS-AD is that it needs a high amount of inoculum to achieve good start-up and efficient biogas production. Despite the potential use of SS-AD for solid biomasses, the comparative studies with W-AD also demonstrated longer digestion times and reduced methane yields due to the higher organic loading rates [10]. In addition, the concentration of lignin and the crystallinity of cellulose limit the biodegradability of lignocellulosic substrates, making the hydrolysis step one of the bottlenecks in anaerobic digestion [11]. The effect of lignocellulosic degradation on methane production has been extensively investigated, but most of the studies are developed for W-AD with TS content under 15% [4]. Since the hydrolysis rate and maximum microbial growth rate are influenced by the water content and the substrate-to-inoculum ratio (SIR), which ultimately affect methane productivity, the inoculation phase becomes a significant step affecting SS-AD, especially for mono-digestion [12].

Therefore, the aim of this study was to investigate the effect of solids loading under wet-AD (10% TS), hemi-solid AD (15% TS), and solid-state AD (20% TS) and compare the results in terms of methane yields, volumetric methane productivity, and kinetic parameters during the batch anaerobic mono-digestion of SCB under mesophilic conditions. The inoculation strategy was also investigated by testing each of the solids loading with increased proportions of the substrate in relation to the inoculum (SIR) o 1, 2, 3, and 4. The results obtained identify the most effective solids loading to support decision-making for the use of SCB for bioenergy production.

2. Materials and Methods

2.1. Substrate and Inoculum

SCB was obtained from the Racecourse Sugar mill (Mackay/Australia). Upon arrival at the laboratory, bagasse was dried at room temperature and milled using a Retsch SM100

ball mill in Germany to attain a homogenous material with a particle size of <20 mm. The prepared material was stored at 4 °C until further use.

The anaerobically digested material from a full-scale plant treating sewage sludge at 37 °C (Queensland Urban Utilities, Brisbane) was used as the inoculum source. At the plant, digested material was separated into solid (cake) and liquid (digestate) fractions by using a decanter centrifuge. A mixture of digestate and solid cake (40:60 w/w) was adopted to achieve a final TS content of 20%. Before use, inoculum was degassed at 37 °C for 7 days to minimize the endogenous methane production. To assess the quality of inoculum, pH, volatile fatty acids (VFAs), and total ammoniacal nitrogen TAN) in the inoculum were determined and compared with values reported elsewhere [13].

2.2. Biochemical Methane Potential Assays

Batch experiments were conducted to determine the biochemical methane potential (BMP) of SCB. BMP assays were carried out in 160 mL glass serum bottles, and headspace was kept in the range of from 48% to 51%. To each assay, SCB and inoculum were added to attain a final TS concentration of 10%, 15%, and 20%, simulating wet, hemi-solid, and solid-state digestion, respectively. Distilled water was used to adjust TS concentration.

Further, the effect of SIR of 1, 2, 3, and 4 based on volatile solids (VS) was determined at each solid loading to explore the optimized substrate load with minimum limitation from hydrolysis. The initial VS concentration of SCB in the bottles ranged from 27 to 38, from 35 to 47, and from 41 to 52 g VS L^{-1} for W-AD, HS-AD, and SS-AD, respectively. Assays with inoculum alone were used as blanks. Microcrystalline cellulose (Sigma Aldrich) was used as a positive control to evaluate the biological activity of inoculum. Assays were sealed with butyl rubber stoppers and aluminum crimps. Headspace in the assays was purged with nitrogen gas (99.9%) for 3 min to create anaerobic conditions. The experiment was conducted in duplicates and incubated at 37 °C. Biogas volume was measured using glass syringes and methane concentration was determined every day during the first 10 days of digestion and thereafter every 2–3 days during the 94 days of digestion until the cumulative methane curve was less than 5% variation for three consecutive measurements. Prior to each gas measurement, the assays were shaken manually for about 1 min. The BMP results were expressed in L CH$_4$/kgVS$_{added}$ and values were converted to standard temperature and pressure (STP).

2.3. Kinetic Study

The kinetic analysis of biogas production was performed by using the first-order kinetic model (Equation (1)) and the modified Gompertz model (Equation (2)). The first-order model was applied assuming hydrolysis to be the rate-limiting step during the anaerobic digestion [14].

$$G(t) = G_0 \times (1 - e^{(-kt)}) \tag{1}$$

G(t): cumulative methane yield at digestion time t (L kg VS^{-1});
G_0: methane potential of the substrate (L kg VS^{-1});
K: methane production rate constant (first-order disintegration rate constant, d^{-1});
t: time (day).

The modified Gompertz model was applied assuming that the biogas production is proportional to the microbial activity following Equation (2).

$$G(t) = G_0 \cdot \exp\left\{-\exp\left[\frac{R_{max} \cdot e}{G_0}(\lambda - t) + 1\right]\right\} \tag{2}$$

R_{max}: maximum methane production rate (L kg VS d^{-1});
λ: lag phase (day);

e: exp (2.7183).

The 'Solver' function in Microsoft Excel (Microsoft 2010) was used to calculate the parameters of the kinetic models using nonlinear regression. The statistical indicators of the kinetic models were evaluated through the coefficient of determination (R^2) and the root mean square error (RMSE) (Equation (3)).

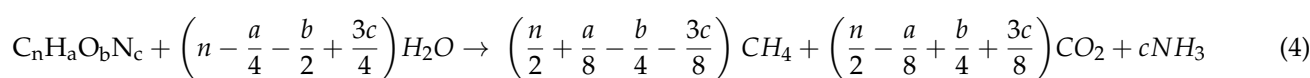$$MSE = \sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}}$$

(3)

n: number of data pairs;
i: ith values;
O: observed methane yield (L kg VS^{-1});
P: predicted methane yield (L kg VS^{-1}).

2.4. Theoretical Methane Potential and Biodegradability

The theoretical methane potential (TBMP) of the SCB was estimated by using Buswell and Mueller [15] as shown in Equations (4) and (5).

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3$$

(4)

$$TBMP\left(\frac{NL\ CH_4}{kg\ VS}\right) = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c}$$

(5)

The biodegradability (BD%) of the SCB was then calculated according to the ratio between the BMP and the TBMP (%) [16].

2.5. Analytical Methods

pH, TS and VS were determined according to Standard Methods [17]. Cellulose, hemicellulose, lignin, and extractives were determined according to the protocol of The National Renewable Energy Laboratory NREL [18]. Elemental compositions (C, N, H, S, O) of substrate were determined according to the method published elsewhere [19]. Soluble and total metal ions, along with the total Kjeldahl phosphorus (TKP) and total Kjeldahl nitrogen (TKN), were analyzed by using an inductivity-coupled plasma–optical emission spectroscopy (ICP-OES) equipped with WinLab32 for ICP software (Perkin Elmer, Waltham, MA, USA Optima 7300 DV) as described elsewhere [20]. Total ammonia nitrogen (TAN) consists of ammonia ion (NH_4^+) and free ammonia (NH_3).

Methane and carbon dioxide concentrations in the biogas were determined simultaneously by a gas chromatograph (GC) (Shimadzu GC-2014, Kyoto, Japan) fitted with a thermal conductivity detector (TCD) and ShinCarbon ST 100/120 packed column (2.0 m length; 1/16″ OD; 1 mm ID). A Volvo GC valve sampling loop (1 mL) was used for injecting the gas sample into the GC. The oven temperature of 110 °C, and detector and injector temperature of 120 °C and 75 °C, respectively, were used. Argon gas was used as the carrier gas, being the sum of the corrected measured values as 100%, assuming that the ammonia and hydrogen sulfide fractions are insignificant quantities (Equation (6)) [16].

$$CH_{4cor}^{dry} = CH_4 \cdot \frac{100}{(CH_4 + CO_2)}$$

(6)

where $CH_{4\ dry,\ cor}$ is the corrected concentration of methane in the dry gas (%), and CH_4 and CO_2 are the measured concentrations of methane and carbon dioxide in the gas (%).

2.6. Statistical Analysis

The triplicate BMP data were submitted to analysis of variance (ANOVA) using IBM SPSS Statistics 26. The differences between the means of the treatments were compared by the Tukey test ($\alpha = 5\%$).

3. Results

3.1. Chemical Composition of Sugarcane Bagasse and Inoculum

Table 1 presents the chemical composition of SCB and inoculum. TS and VS content of the inoculum were 13.6% and 9.8%, respectively (Table 1). The VS/TS ratio of the inoculum was 71.8%, greater than the 50% recommended by the BMP protocol [21]. pH, VFA, and ammonia concentration of the inoculum were 7.9, 1.1 gCH_3COOH L^{-1}, and 2.0 $gN-NH_4$ L^{-1}, respectively. All these parameters were in concentrations that are within the optimal ranges of from pH 7.0 to 8.5; VFA ≤ 1.0 gCH_3COOH L^{-1} and ammonia < 2.5 $gN-NH_4$ L^{-1}, as reported by Holliger et al. [13]. The C/N ratio of the inoculum was 6.9, indicating a good buffering capacity and a nitrogen concentration that is sufficient for microbial growth in the batch tests.

Table 1. Physical and chemical characterization of sugarcane bagasse.

Parameter	Sugarcane Bagasse	Inoculum
TS (%)	82.9 ($\pm$0.2)	13.6 ($\pm$0.3)
VS (%)	75.6 ($\pm$0.9)	9.8 ($\pm$0.0)
pH	N.D.	7.9 ($\pm$0.1)
Glucan (%)	28.5 ($\pm$0.8)	N.D.
Xylan (%)	15.6 ($\pm$0.7)	N.D.
Arabinan (%)	2.3 ($\pm$0.0)	N.D.
Acid insoluble lignin (%)	17.7 ($\pm$0.7)	N.D.
Acid soluble lignin (%)	4.5 ($\pm$0.0)	N.D.
Ash (%)	6.2 ($\pm$0.3)	N.D.
Extractives (%)	2.5 ($\pm$0.0)	N.D.
C (%)	42.7 ($\pm$0.7)	36.8 ($\pm$N.D.)
N (%)	0.4 ($\pm$0.0)	5.6 ($\pm$N.D.)
H (%)	5.3 ($\pm$0.1)	N.D.
S (%)	0.0 ($\pm$0.0)	N.D.
O (%)	31.4 ($\pm$1.3)	N.D.
C/N	106.8	6.9

TS: total solids; VS: volatile solids. Each value represents mean $\pm$ SD of three replications. Percentage results are expressed in dried basis (% TS).

The TS and VS contents of the SCB were 82.9% and 75.6%, respectively. Similar values of TS of 87.7% and VS of 81.0% for SCB were reported in the literature [22,23]. The high VS/TS ratio of 91.2% indicated that SCB is high in organic material. However, lignocellulose content (mono- and disaccharides and lignin) accounted for 68.6% of the feedstock VS, suggesting that a high concentration of feed VS contains less degradable organic matter. This was also indicated by the very high C/N ratio of 106.8, when compared to the optimal range of 20–30 for biological treatments [24]. Extractives accounted for 2.5% of VS of the SCB. This was lower than the value of 5.1–35.1 reported for different lignocellulosic substrates [4]. The very low content of extractives in the SCB, when compared to other biomass streams, indicates limited availability of easily degradable organic matter, thereby leading to low daily biogas production, especially in the first days of digestion. In general, extractives are composed of easily degradable free sugars, oligosaccharides, and organic acids [25]. These extractives can be degraded and potentially be converted into biogas in a relatively short time.

3.2. Effect of Different Solids Concentration on Methane Yields from SCB

The endogenous methane production from inoculum was 55 ± 9 L CH_4 kgVS^{-1} added. This result was in accordance with the recommended value of approximately 50 L CH_4 kgVS^{-1} added reported in the literature [13]. This result indicates that the inoculum in the present study has been degassed sufficiently and only a small amount of residual substrate was left. The methane yield of microcrystalline cellulose, used as a positive control, was 517 ± 7 L CH_4 kgVS^{-1} added. This experimental methane yield was 80% of the theoretical methane potential (375 L CH_4 kgVS^{-1} added) after 30 days of digestion [21]. This result demonstrates that the biological activity of the inoculum was adequate.

3.2.1. Wet Anaerobic Digestion (W-AD)

Figure 1 shows the effect of SIR on the methane production from SCB at 10% solids (W-AD). The results showed that biogas production in all W-AD assays started after a lag phase of 1 day (Figure 1). However, SIR had a profound influence on the methane production rates and cumulative methane yields from SCB under W-AD conditions. An increase in SIR from 2 to 4 resulted in delayed and lower mean methane production rates than at SIR 1. For instance, the highest daily methane production rate of 10 L CH_4 kgVS^{-1} d^{-1} was obtained after 1 day of digestion at SIR 1 (Figure 1d). On the other hand, the highest daily methane production rates of 5, 4, and 4 L CH_4 kgVS^{-1} d^{-1} were achieved after 3, 10, and 13 days of digestion for SIR 2, 3, and 4, respectively. The lower and delayed methane production rates observed when the SIR was increased can be explained by the reduction in the proportion of pertinent microbes in the inoculum in relation to the substrate for W-AD.

Similarly, an increase in SIR for W-AD also resulted in a decrease in cumulative methane yields. Mean cumulative methane yields were 125, 97, 85, and 71 L CH_4 kgVS^{-1} added, obtained at SIR 1, 2, 3, and 4, respectively (Table 2). The methane yields obtained in the present study were lower than those reported in the literature. Bolado-Rodríguez et al. [26] and Liu et al. [27] reported methane yields of 222 and 248 L CH_4 kgVS^{-1} added for the same biomass. The difference in the SIR used in the above studies is the possible explanation for this discrepancy. For instance, the SIR used in the above studies was the traditional SIR of 0.5 used for W-AD compared to the SIR of 2 used in the present study. Moreover, solid loading in W-AD experiments is generally not reported. However, it should be noted that the solid content in the inoculum obtained from full-scale biogas plants treating either animal manure or sewage sludge is lower than 2–3% (dry wt). The W-AD tested with SIR 1 and 10% TS in this study was used to guarantee comparable results with H-AD (15% TS) and SS-AD (20% TS).

The T_{90} (time to achieve at least 90% of the cumulative methane yield) was calculated to be 66 days for all investigated SIR under W-AD (Table 2). The technical digestion time T_{90} can be used to indicate the hydraulic retention time (HRT) in full-scale biogas plants for this type of substrate [14]. Edwiges et al. [28] reported only 15 days of T_{90} for garden waste used as lignocellulosic feedstock under W-AD (TS 2%) and at a SIR of 0.33. The relatively higher T_{90} obtained for the W-AD of SCB in this study can be related to the higher TS concentration in the batch test, much higher SIR, and also the different chemical composition of SCB, with more lignocellulose content than typical garden waste.
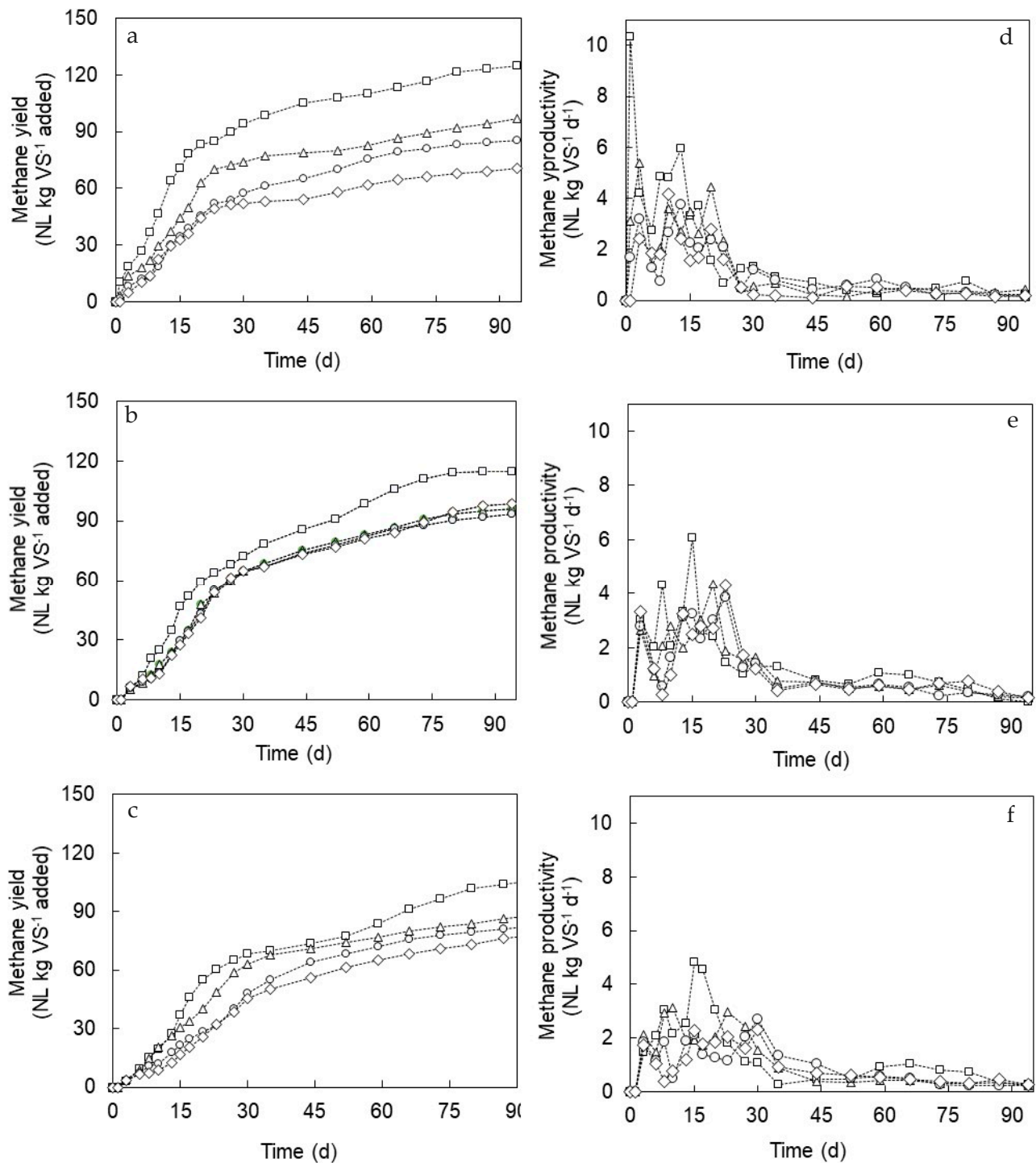
Figure 1. Methane yield from sugarcane bagasse under W-AD (TS 10%) (**a**), HS-AD (TS 15%) (**b**), and SS-AD (TS 20%) (**c**), and at different ISR: 1 (□), 2 (△), 3 (○), and 4 (◇). Daily methane yield from sug-arcane bagasse under W-AD (**d**), HS-AD (**e**), and SS-AD (**f**).

Table 2. Cumulative biogas and methane yield from sugarcane bagasse at different operational conditions.

Operational Condition	TS (%)	VS from Feedstock (%)	SIR	Biogas Yield (L kg VS^{-1})	Methane (%)	Methane Yield (L kg VS^{-1})	T$_{90}$ (Day)
Wet	10	4.0	1	223 ($\pm$23)	56 ($\pm$0)	125 ($\pm$13) [a]	52
		5.6	2	176 ($\pm$16)	55 ($\pm$1)	97 ($\pm$11) [bcde]	59
		6.4	3	155 ($\pm$2)	55 ($\pm$0)	85 ($\pm$1) [cde]	59
		6.9	4	131 ($\pm$2)	54 ($\pm$0)	71 ($\pm$1) [e]	59
Hemi-solid	15	6.0	1	217 ($\pm$21)	53 ($\pm$0)	115 ($\pm$11) [ab]	66
		8.4	2	184 ($\pm$18)	51 ($\pm$1)	94 ($\pm$9) [bcde]	59
		9.6	3	180 ($\pm$6)	52 ($\pm$3)	93 ($\pm$3) [bcde]	59
		10.4	4	190 ($\pm$15)	52 ($\pm$2)	99 ($\pm$8) [bcd]	66
Solid-state	20	8.0	1	200 ($\pm$13)	53 ($\pm$0)	106 ($\pm$7) [abc]	66
		11.0	2	160 ($\pm$4)	55 ($\pm$0)	88 ($\pm$2) [cde]	59
		12.8	3	153 ($\pm$6)	54 ($\pm$0)	82 ($\pm$3) [cde]	59
		13.7	4	153 ($\pm$4)	51 ($\pm$2)	78 ($\pm$2) [de]	59

TS: total solids; VS: volatile solids; SCB: sugarcane bagasse; SIR: substrate to inoculum ratio; SD ($\pm$standard deviation). TS (%) is expressed on the total solids of the batch bottle, while VS represents the volatile solids concentration from SCB in the batch bottles. T$_{90}$: time to achieve 90% of the methane yield. Means followed by the same letter do not differ significantly at 5% probability by Tukey test.

3.2.2. Hemi-Solid Anaerobic Digestion (H-AD)

The results of the methane production rates and yields during the H-AD are presented in Table 2. The results showed that the biogas production from SCB under H-AD started after a lag phase of 3 days. This long delay in biogas production in H-AD when compared to W-AD (1 day) was obviously due to the increase in the TS concentration from 10% to 15%. The peak in the daily methane production rate at SIR 1 was 6 L CH$_4$ kg VS$_{-1}$ d^{-1}, and it was obtained after 15 days of digestion. On the other hand, methane production rates of 4 L CH$_4$ kg VS^{-1} d^{-1} were noticed after 20 days of digestion at SIR, 2, 3, and 4 (Figure 1e). These results indicate that, similar to W-AD, SIR 1 was the best operational condition for H-AD. In addition, an increase in SIR to more than 1 resulted in delayed methane production regardless of the adopted operational TS (%) (Table 2).

The cumulative methane yields of the SCB under H-AD were 115, 94, 93, and 99 L CH$_4$ kg VS^{-1} for SIR 1, 2, 3, and 4, respectively (Table 2). Increasing the relative amount of inoculum by decreasing SIR resulted in higher methane yields due to the more stable process. However, the increased amount of inoculum (SIR < 1) can possibly result in high CAPEX and low economic gain due to unnecessarily increased reactor size, use of low feed rate, and higher energy demand and digestate production. When comparing the best results from both W-AD and H-AD (SIR 1), an increase in 50% of the operational TS (10% to 15%) resulted in only an 8% decrease in methane yields (125 and 115 L CH$_4$ kg VS^{-1}, respectively). Increasing operational TS with similar methane potential is advantageous for the economic viability of real-scale biogas plants, demanding smaller reactor sizes and also lower demand for energy and water input and digestate management. Methane concentration in the biogas ranged from 51% to 53% for H-AD, and it was slightly lower than the values of from 54% to 56% for W-AD. The T90 for H-AD was 66 days at SIR 1, 2, and 3 (similar to W-AD) and 73 days at SIR 4.

3.2.3. Solid-State Anaerobic Digestion (SS-AD)

Methane production rates and yields under SS-AD conditions are presented in Table 2. The results showed that methane production rates under SS-AD were similar to those noticed under H-AD. A 3-day lag phase noticed under SS-AD suggests that an increase in solids content from 15% to 20% had no negative impact on the initial degradation of the organic matter. Similar to W-AD and H-AD, SIR 1 was the best operational condition for SS-AD. Peak daily methane production rates of 5 L CH$_4$ kg VS^{-1} d^{-1} were obtained after

15 days of incubation. This was similar to the methane production rates obtained under H-AD conditions. The corresponding values for SIR 2, 3, and 4 were 2–3 L CH$_4$ kg VS^{-1} d^{-1} obtained after from 10 to 30 days of digestion (Figure 1f). Brown et al. [4] measured the maximum daily methane production rates from several lignocellulosic feedstocks, including wheat straw, yard waste, and switchgrass under SS-AD, and reported similar values in the range of 2.5–12 10 L CH$_4$ kg VS^{-1} d^{-1} after from 4 to 13 days of digestion. Adarme et al. [1] also described the negative effect of increasing SIR, reporting lower methane yields when higher amounts of lignocellulosic substrate were available to microorganisms.

The cumulative methane yields of 106, 88, 82, and 78 L CH$_4$ kg VS$_{-1}$ were obtained at SIR 1, 2, 3, and 4, respectively (Table 2). Similar methane yields ranging from 85.6 to 143.3 L CH$_4$ kg VS^{-1} from SCB were reported when 15% TS and SIR 2 were used to simulate the SS-AD conditions [29]. The relatively low methane yields from lignocellulosic biomass under SS-AD compared to traditional feedstocks like animal manure with methane yield ranging from 155 to 287 L CH$_4$ kg VS^{-1} [30] or food waste in the range of 220–440 L CH$_4$ kg VS^{-1} [31] are attributed to the recalcitrance of the plant cell wall to digestion, which, together with the low moisture content, limited the hydrolysis and biodegradability of the lignocellulosic biomass. However, the methane concentration of from 51% to 53% in the biogas indicated no inhibition in terms of methanogenic activity during SS-AD.

3.2.4. Comparison Between W-AD, H-AD, and SS-AD

A comparison of the effect of solids content on methane yields under W-AD, H-AD, and SS-AD is presented in Figure 1. The results showed that methane concentration increased during the initial days of digestion and stabilized after 13, 15, and 17 days for W-AD, H-AD, and SS-AD, respectively. However, methane yields of <50 L CH$_4$ kg VS^{-1} were obtained under SS-AD. A similar result was reported by Motte et al. [12], which can represent only 10% of the theoretical methane yield [32]. The theoretical methane yield (TMY) of the SCB used in the present study, calculated according to Equations (5) and (6), was 545 L CH$_4$ kg VS^{-1}. The calculated biodegradability values of the SCB, estimated as the ratio of experimental to the theoretical methane yield (%) obtained at SIR 1, were 22.9%, 21.1%, and 19.4% for W-AD, H-AD, and SS-AD, respectively.

The low biodegradability of the SCB with a relatively long incubation time of 94 days to attain maximum cumulative methane yields indicates that microorganisms need a long period of adaptation to degrade under SS-AD conditions. Jansson et al. [33] also reported an extended experimental time of 112 days until the cumulative methane yields ceased during the SS-AD of paper waste. Both these results indicate that longer retention times are required for the acclimatization of microorganisms to avoid process failure when hard-degradable feedstocks are used under SS-AD conditions. Increasing the SIR from 1 to 4 also increased the initial C/N of the assays from 57 to 73, 82, and 87, respectively.

The increase in C/N with increased SIR might have limited the availability of nitrogen, which is crucial for microbial growth. This was evident from the long retention time needed to achieve the maximum cumulative methane yields in this experiment. In practice, co-digestion of SCB with nitrogen-rich co-substrates such as livestock manure can potentially decrease the C/N and overcome the lack of nitrogen and the significant costs associated with urea addition in commercial-scale biogas plants. Moreover, the availability of easily degradable carbohydrates in livestock manure may also facilitate improving the methane production rates, especially during the critical initial days of digestion, thereby allowing for the maintenance of a satisfactory concentration of methanogenic bacteria in the biogas plant.

The methane yields of the SCB obtained under SS-AD at SIR 1 were 15% and 8% lower than those obtained under W-AD and H-AD, respectively. However, no statistical difference was noticed between the methane yields regardless of the operational TS con-

centration. In addition, SS-AD allowed an increase in terms of TS concentration of 50% and 100% compared to H-AD and W-AD, respectively. Similarly, no significant differences between W-AD and SS-AD were reported by Brown et al. [4] using several lignocellulosic feedstocks. Lower methane yields in SS-AD than in H-AD and W-AD may be attributed to the imbalances of hydrolytic, fermentative, and acetogenic bacteria and methanogenic archaea [4]. The T_{90} values in the present study were reported to be between 52 and 66 days for all tested SIRs studied under SS-AD, which were similar to those obtained for W-AD and SS-AD.

The volumetric methane productivities of SCB under the studied conditions are presented in Figure 2. The volumetric methane productivities were expressed as m^3 CH_4/m^3 work. The word "work" here is denoted as the total mass of feedstock, inoculum, and water to simulate potential real-scale reactor sizes. Overall, volumetric methane productivity increased with increased TS loading. The volumetric methane productivities for SCB with SIR 1 were 3.39, 4.01, and 4.35 m^3 CH_4/m^3 work under W-AD, H-AD, and SS-AD conditions, respectively. As expected, volumetric methane yields for H-AD and SS-AD were 118% and 128% higher than the yield obtained for W-AD, respectively. Higher volumetric methane productivity noticed for SS-AD suggests an operational and economic advantage on a commercial scale as it requires smaller reactor sizes [4].
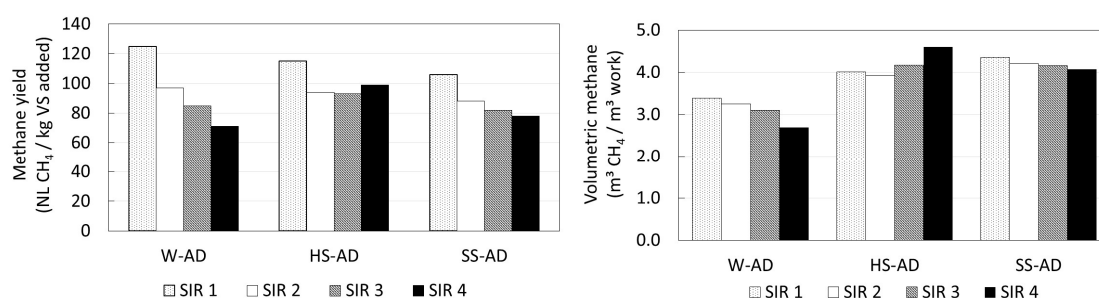

Figure 2. Comparison between methane yield (**left**) and volumetric methane (**right**) under different TS and SIR.

3.3. Kinetic Parameters

The cumulative methane yields obtained were simulated by using first-order and modified Gompertz models to analyze the best solids loading and SIR that would provide the maximum methane production. Kinetic parameters such as hydrolysis rate constant, maximum methane production rate, lag phase, and methane yield were determined to evaluate the fitness of these models (Table 3). The predicted and experimental methane yields using first-order and modified Gompertz models are presented in Table 3. Overall, the first-order model was shown to be the best fit. The percentage difference between the predicted and experimental BMP values for the order model was low (1–5%), along with low RMSE with errors < 4.5 L CH_4 kg VS^{-1}.

The corresponding values for the modified Gompertz model were in the range of 4–11% difference in methane yields between predicted and experimental methane yields and RMSE value of 6.3 L CH_4 kg VS^{-1}. The percentage difference between the experimental and predicted methane yields for SS-AD (SIR from 2 to 4) was lower when the modified Gompertz model was used (differences < 6%) than with the first-order kinetic model (<11%). The average RMSE values were 2.0 and 2.3 L CH4 kg VS−1 using the modified Gompertz model and first-order kinetic models, respectively. The possible reason for the first-order model being the best fit for W-AD and H-AD rather than the modified Gompertz is that the first-order kinetics model determines the cumulative methane production and hydrolysis rate constant (K_{hyd}). The latter parameter is the crucial kinetic parameter, and, in the

anaerobic digestion of lignocellulosic feedstock, hydrolysis is considered the rate-limiting reaction. On the other hand, the modified Gompertz model provides information on the influence of bacterial growth. This is evident from the fact that the modified Gompertz model fitted well for assays with SS-AD. This is obvious as the modified Gompertz model is dependent on the bio-kinetic parameters and was evident by lower methane production with a longer lag phase due to an increase in substrate concentration (Table 2). The higher the substrate concentration, the longer the hydrolysis phase would be. The solids loading in W-AD and H-AD-solid conditions (VS 4–10%) were lower than SS-AD (11–14%) (Table 2).

Table 3. Summary of results from kinetic study using first-order and modified Gompertz model.

Parameter	TS 10 SIR 1	TS 10 SIR 2	TS 10 SIR 3	TS 10 SIR 4	TS 15 SIR 1	TS 15 SIR 2	TS 15 SIR 3	TS 15 SIR 4	TS 10 SIR 1	TS 10 SIR 2	TS 10 SIR 3	TS 10 SIR 4
BMP (L kg VS^{-1})	125	97	85	71	115	94	93	99	106	88	82	78
BMP$_1$ (L kg VS^{-1})	120	92	87	68	120	96	94	100	105	88	92	83
Difference (%)	4	5	−2	4	−4	−2	−1	−1	1	0	−11	−6
K$_{hyd}$ (d^{-1})	0.05	0.05	0.04	0.05	0.03	0.04	0.04	0.04	0.04	0.04	0.03	0.03
Time delay (d)	0.0	1.4	1.6	2.2	1.8	4.6	5.0	4.6	2.7	4.0	4.5	6.4
R^2	0.990	0.984	0.993	0.988	0.994	0.995	0.993	0.987	0.984	0.994	0.994	0.994
RMSE	3.9	4.0	2.4	2.6	3.1	2.5	2.8	3.8	4.5	2.4	2.2	2.2
BMP$_2$ (L kg VS^{-1})	114	88	81	64	110	90	88	91	96	83	81	75
Difference (%)	10	10	5	11	5	4	6	9	10	6	1	4
Rm (L kg VS^{-1} d^{-1})	4.4	3.2	2.2	2.3	2.7	2.6	2.6	2.5	2.6	2.4	1.8	1.7
λ (d)	0.0	0.8	0.6	1.4	0.4	3.4	4.0	3.5	1.8	2.8	4.1	5.1
R^2	0.978	0.984	0.985	0.978	0.979	0.992	0.991	0.984	0.968	0.993	0.998	0.995
RMSE	5.7	3.9	3.5	3.5	5.6	3.0	3.1	4.3	6.3	2.6	1.5	2.0

BMP$_1$: predicted BMP using first-order model; BMP$_2$: predicted BMP using modified Gompertz model; k: first-order disintegration rate constant; Rm: maximum methane production rate; λ: lag-phase; R^2: coefficient of determination; RMSE: root square mean error.

The hydrolysis constant rate (K$_{hyd}$) for all studied treatments was in the range of 0.04–0.05 d–1 for W-AD, 0.03–0.04 d–1 for H-AD, and 0.03–0.04 d–1 for SS-AD (Table 3). The slightly better W-AD conversion constants than those obtained for H-AD and SS-AD were possibly due to better mixing and homogeneous conditions. The K$_{hyd}$ values obtained in the present study are lower or comparable to the values reported in the literature. For instance, K$_{hyd}$ values of 0.028–0.1 for W-AD (5.1–5.6% TS), 0.009–0.129 for H-AD (10.1–11.2% TS), and 0.007–0.071 for SS-AD (20.1–22.4% TS) were reported during the co-digestion of corn stover (CS) and chicken manure (CM) in batch assays at CS:CM ratios of 1:0, 3:1, 1:1, 1:3, and 0:1 (based on VS) and SIR of 3, on a VS basis [5]. On the other hand, Brown et al. [4] reported slightly higher K$_{hyd}$ values ranging from 0.08 to 0.09 for W-AD (5% TS) and from 0.09 to 0.35 for SS-AD (18–19% TS) using lignocellulosic biomass (switchgrass, corn stover, wheat straw, yard waste, leaves, waste paper, maple, and pine feedstock). Paulose and Kaparaju [23] reported a K$_{hyd}$ value of 0.056 d-1 for SCB at a SIR of 0.5 on a VS basis. Similarly, Edwiges et al. [28] reported K$_{hyd}$ values of 0.59 for fruit and vegetable waste and 0.19 for garden waste under W-AD (2% TS) and at SIR of 0.33. The discrepancy in K$_{hyd}$ values in the present study with the literature could be due to differences in the substrate composition.

The presence of fast and slow digestible components, for example, easily digestible extractives and relatively resistant structural carbohydrates in substrates may lead to two or more conversion rates during the AD process. For instance, very low concentrations of simple sugars and the large lignocellulosic content of the SCB might result in slow degradation of the organic matter and methane production during the initial days of digestion. This was evident from the time delay, which was affected by TS concentration, with an average of 1.3, 4.0, and 4.4 days for W-AD, H-AD, and SS-AD and was imposed due to lower moisture conditions. The increase in the SIR for each operational TS resulted in a significant increase

in time delay, with Pearson's linear correlation coefficient (r) between SIR and time delay is 0.94, 0.77, and 0.98 for W-AD, H-AD, and SS-AD, respectively. Nevertheless, no significant changes were observed for the hydrolysis rate when TS concentration was increased from 10% to 20%. This is obvious as SCB particles settled to the bottom of the assay may have created local areas of a much higher SIR ratio than desired and led to a shortage of microbes to break down the particles. This is evident from the total volatile fatty acids (TVFAs) in the assays.

3.4. Process Stability and Solids Removal

Process stability and methane yields depend on the process parameters and operation conditions. An imbalance in AD is generally caused by improper feedstock solids concentration, low pH, accumulation of VFAs, C/N ratio, and high total ammonia–nitrogen and free ammonia concentrations [19]. The total VFA concentration in the assays at the end of 35 and 94 days of digestion is presented in Figure 3. Overall, total VFA concentration increased with increasing TS content and decreased with digestion time. After 35 days of digestion, total VFA concentration was, on average, 980 ± 295 mg L^{-1} in W-AD, 753 ± 129 mg L^{-1} in H-AD, and 727 ± 100 mg L^{-1} in SS-AD (Figure 3a).
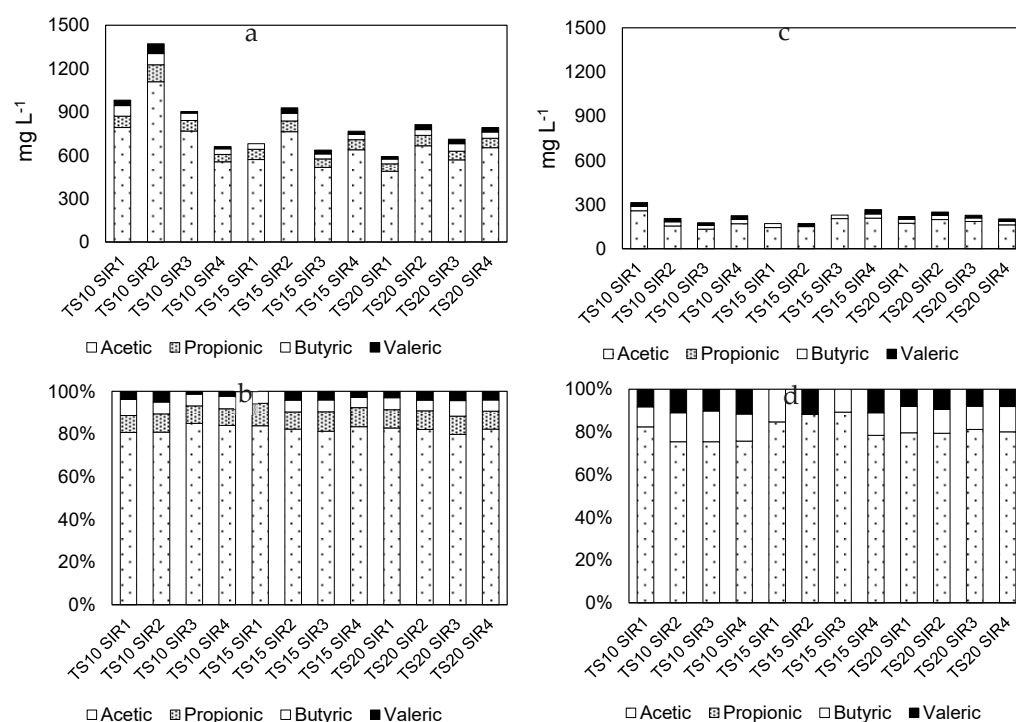

Figure 3. Absolute and relative concentration of volatile fatty acids at 35 (**a**,**b**) and 94 (**c**,**d**) days of digestion.

The high dispersion of data at 35 days (coefficient of variation (CV) = 26%) indicated the impact of TS concentration and SIR on the stability of the anaerobic digestion. In addition, the higher total VFA in W-AD, when compared to SS-AD, indicates its more intense biodegradation rates in the first period of digestion due to the higher water availability. Acetic acid was the most abundant VFA component and accounted for 80–85% of the total VFA at 35 days for SIR 1, 2, 3, and 4 (Figure 3b). Propionic, butyric, and valeric acids accounted for 8–10%, 5–8%, and 0–5% of the remaining VFA, respectively. No significant variations of the VFA proportions were observed with the different SIR. However, the intense bacterial activity and higher proportions of acetic acid, even at 35 days of digestion, were in accordance with the higher T_{90} values obtained for all treatments.

The total VFA concentration decreased along the digestion time, reaching 230 ± 59 mg L^{-1} in W-AD, 208 ± 47 mg L^{-1} in H-AD, and 224 ± 19 mg L^{-1} in SS-AD at 94 days (Figure 3c). The average VFA concentration at 94 days (221 ± 42 mg L^{-1}) was 73% lower than the average concentration noticed at 35 days of digestion (820 ± 212 mg L^{-1}), indicating the efficient conversion of VFA into methane with longer digestion times. Acetic acid was the most abundant VFA, accounting for 75–89% of the total VFA (Figure 3d). The corresponding values for butyric and valeric acid were 0–15% and 0–12% of total VFA, respectively. On the other hand, propionic acid was not detected in the assays at 94 days of digestion.

Ammonia nitrogen (NH_3) concentration after 35 days of digestion was 883, 1050, and 1190 mg L^{-1} for W-AD, H-AD, and SS-AD, respectively (Figure 4). The increase in the concentration of ammonia with an increase in TS can be related to higher VS content of SCB added to each operational condition (Table 2). In addition, the concentration of ammonia was inversely correlated to the SIR at all tested TS concentrations (Figure 4). A strong and negative Pearson's linear coefficient correlation of –0.972, –0.999, and –0.992 was noticed between SIR and solids content for W-AD, H-AD, and SS-AD, respectively. An increase in SIR from 1 to 2 resulted in the concentration of NH_3 decreasing by 8% for each TS condition. A further increase in SIR to 3 and 4 showed a 37% reduction in ammonia concentration. An increase in the SIR limited the anaerobic degradation of the SCB, resulting in lower formation of ammonia during the acidogenesis phase. In addition, the high content of nitrogen after day 35 indicates the slow degradation of the organic matter even after 30 days of digestion.
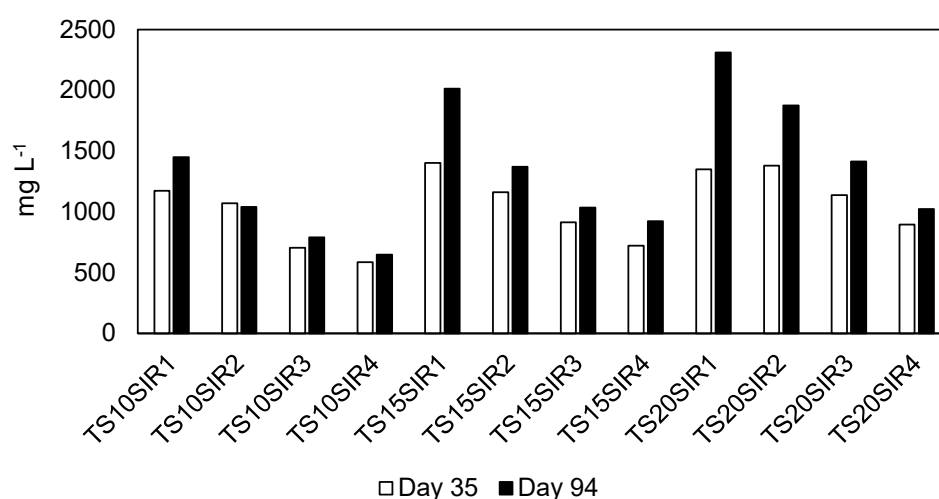

Figure 4. Ammonia concentration (NH_4) at day 35 and 94 days of digestion.

The concentration of ammonia increased from day 35 to 94 in all assays (Figure 4). The mean ammonia concentrations were 981, 1336, and 1655 mg L^{-1} for W-AD, H-AD, and SS-AD, respectively. This increase in ammonia concentration was proportional to the increase in the TS. In comparison to day 35, ammonia concentrations were increased by 11%, 26%, and 36% on day 94 for W-AD, H-AD, and SS-AD, respectively. Similar to day 35, the correlation between ammonia and SIR was strongly negative, with Pearson's coefficient correlation being –0.976, –0.950, and –0.999. The high concentrations of ammoniacal nitrogen, even at 94 days of digestion, indicate the efficiency of the hydrolysis phase and accumulation under the inhibition levels. The concentration of NH_3 was reported to inhibit methanogen activity when it exceeds 3000 mg/L [33], which was not the case in this study. In addition, the high nitrogen content in the digestate after digestion suggests the potential to reuse it as a biofertilizer for crop production. Lower methane yields in SS-AD than in

H-AD and W-AD may be attributed to the imbalances of hydrolytic, fermentative, and acetogenic bacteria, and methanogenic archaea [4].

A VS reduction at the end of 35 and 94 days of digestion is presented in Figure 5. The VS reduction increased with digestion time. After 35 days of incubation, the VS reduction ranged from $9.9 \pm 2.8\%$ in W-AD, $6.0 \pm 4.2\%$ in H-AD, and $9.5 \pm 4.0\%$ in SS-AD. The corresponding values after 94 days were $17.9 \pm 1.3\%$, 23.5 ± 2.2 and $19.5 \pm 0.8\%$ (Figure 5). The low VS removal noticed after 30 days of digestion is related to the low biodegradability (BMP/TBMP) of the SCB. As expected, the VS removal increased (140% average) from day 35 to day 94 as microbes had more time to adapt to the conditions and digest the less degradable organic matter in SCB. The highest VS removal was achieved when SCB was incubated at SIR 1, followed by SIR 2, 3, and 4.
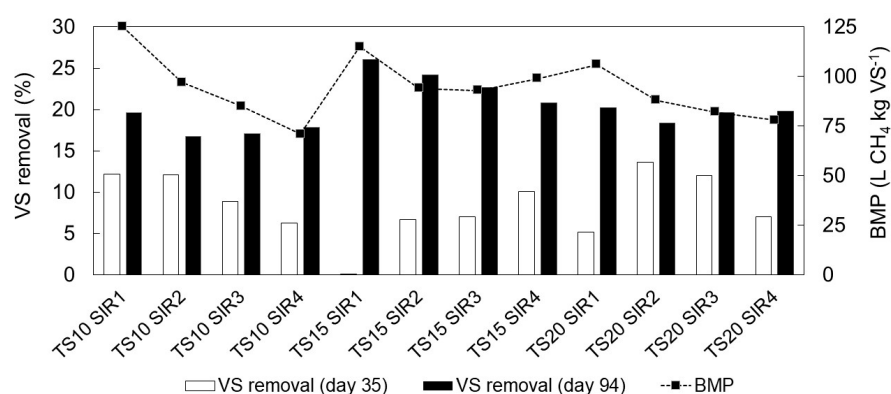

Figure 5. VS removal (%) after 35 and 94 days for W-AD, HS-AD, and SS-AD.

The results of the present study show that solid loading and SIR have a profound influence on hydrolysis, volumetric methane production rates, methane yields, and process stability. The high VS removal rates with lower VFA and ammonia concentration at W-AD conditions than at H-AD and SS-AD indicate that moisture content in the latter AD conditions was the limiting factor for the hydrolysis of the lignocellulosic biomass. This low moisture content and poor contact between substrate and microorganisms might have exacerbated the overall mass transfer, resulting in an accumulation of dissolved hydrogen, dissolved methane, and transfer of dissolved carbon dioxide, leading to an inhibitory effect on hydrolysis and methanogenesis.

For instance, the CO_2 produced during methanogenesis becomes trapped within the substrate matrix, leading to increased CO_2 concentration and local acidification. This localized acidification might have inhibited methanogenesis, leading to VFA accumulation. Similarly, the accumulation of dissolved hydrogen may also cause further degradation of VFAs, leading to the accumulation of valerate, butyrate, and propionate in the assays. Thus, a good liquid/gas mass transfer and the syntrophic association between hydrogen-producing acetogens and hydrogen-consuming hydrogenotrophic methanogens are required in order to promote very low hydrogen partial and prevent the accumulation of VFAs.

This was evident from the high methane content, low methane yields, and high volumetric methane yields for H-AD and SS-AD compared to W-AD, respectively. Higher volumetric methane productivity noticed for SS-AD suggests an operational and economic advantage on a commercial scale as it requires smaller reactor sizes, low energy requirements for heating and/or mixing, minimal material handling, and positive energy balances. The low VS removal under SS-AD means that it will also reduce the energy requirements for digestate handling and facilitate composting of the high solids digestate to recycle the nutrient.

4. Conclusions

SCB is a carbon-rich and hardly degradable substrate, composed of approximately 70% VS of lignocellulose, that can be transformed into bioenergy using a dry-digestion strategy and improve the sustainability and circularity of the sugar and ethanol industry. This study highlights the critical influence of substrate-to-inoculum ratios (SIR) and solids concentrations on methane yields and volumetric productivity. The SIR of 1 resulted in the highest methane yields of 125, 115, and 106 L CH_4 kg VS^{-1} for W-AD (10% TS), H-AD (15% TS), and SS-AD (20% TS). Increasing the SIR from 1 to 4 resulted in decreased methane yields for all TS conditions and no statistical difference was observed between methane yields with the same SIR. Instead, volumetric productivities from H-AD and SS-AD were 118% and 128% higher than the yields obtained under W-AD. These findings provide actionable insights for the design of commercial-scale biogas facilities since SS-AD requires smaller reactor sizes and requires less energy for the management of digestate. For future studies, we recommend evaluating the microbiological composition of the inoculum and digestate at the end of the treatment, as well as the application of pretreatments to increase the biodegradability of the lignocellulose present in the bagasse.

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