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Sweet Sorghum as a Potential Fallow Crop in Sugarcane Farming for Biomethane Production in Queensland, Australia

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Abstract: Biogas from lignocellulosic feedstock is a promising energy source for decentralized renewable electricity, heat, and/or vehicle fuel generation. However, the selection of a suitable energy crop should be based on several factors such as biomass yields and characteristics or biogas yields and economic returns if used in biorefineries. Furthermore, the food-to-fuel conflict for the use of a specific energy crop must be mitigated through smart cropping techniques. In this study, the potential use of sweet sorghum as an energy crop grown during the fallow periods of sugarcane cultivation was evaluated. Nine sweet sorghum cultivars were grown on sandy loam soil during September 2020 in North Queensland, Australia. The overall results showed that the crop maturity had a profound influence on chemical composition and biomass yields. Further, the total insoluble and soluble sugar yields varied among the tested cultivars and were dependent on plant height and chemical composition. The biomass yields ranged from 46.9 to 82.3 tonnes/hectare (t/ha) in terms of the wet weight (*w*/*w*) of the tested cultivars, with the SE-81 cultivar registering the highest biomass yield per hectare. The gross energy production was determined based on the chemical composition and methane yields. Biochemical methane potential (BMP) studies in batch experiments at 37 °C showed that methane yields of 175 to 227.91 NmL CH_4/gVS_{added} were obtained from the tested cultivars. The maximum methane yield of 227.91 NmL CH_4/gVS_{added} was obtained for cultivar SE-35. However, SE-81 produced the highest methane yields on a per hectare basis (3059.18 Nm^3 CH_4/ha). This is equivalent to a gross energy value of 761.74 MWh/year or compressed biomethane (BioCNG) as a vehicle fuel sufficient for 95 passenger cars travelling at 10,000 km per annum. Overall, this study demonstrated that sweet sorghum is a potential energy crop for biogas production that could be cultivated during the fallow period of sugarcane cultivation in Queensland.

Keywords: sweet sorghum; anaerobic digestion; energy production; kinetic modelling; biomethane

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1. Introduction

Lignocellulosic feedstocks have excellent potential in the biorefinery sector, as they are the largest source of carbohydrates for biofuel production [1]. However, the large-scale application of energy-based feedstocks is limited due to the food-to-fuel competition and lack of arable land for cultivation [2]. Some of the main energy crops grown worldwide for biofuel production are wheat, barley, corn, sugarcane, and rice [3]. These crops are also considered essential food sources and are expensive to grow on a broad acreage due to competition between food and fuel [4].

Globally, 1.3 billion hectares (ha) of land is suitable for growing food crops [5]. According to the World Biogas Association (WBA), if 7% of the above agricultural land is used for the

sustainable cultivation of energy crops, there is the potential to generate 3350 to 5000 terra watt hour (TWh) of biogas [6]. However, the estimated global energy potential from sustainably grown/recovered biomass is between 10,100 and 14,000 TWh [7]. This energy can meet 6–9% of the global primary energy consumption or 23–32% of the global coal consumption [8]. The development of several biofuels from energy crops can be a way to increase energy efficiency and reduce its overall cost of production. By substituting for fossil-fuel-based energy, sustainably grown energy crops have the potential to offset 910 to 1350 Mt of the carbon dioxide equivalent (CO_2 eq.) of greenhouse gas (GHG) emissions [9]. In addition, the recent increase in gas and oil prices for industries including transportation and agricultural machinery can be mitigated by using the biogas produced from the energy crops [5].

Sweet sorghum (*Sorghum vulgaris* [L.] Moench), which is rich in carbohydrates [10], is a drought-tolerant crop that can thrive on marginal soils with low nutrient requirements. In addition, it can produce a large amount of biomass that is rich in fermentable sugars [11,12]. Its high biomass yields per hectare (ha) and short growth period compared with other commercial energy crops, such as maize, wheat, corn, and sugarcane [13–15], makes it as an ideal multipurpose crop that can be used for food, fodder, and energy production [16]. In order to avoid the food-to-fuel conflict, sweet sorghum can be planted on marginal areas and rotated with other legume or cereal crops [17] or even grown as an energy crop during the fallow period of annual crops [18].

The use of fallow land to grow non-food crops for bioenergy production has been shown to compensate for the variable costs incurred during food crop cultivation [19]. Currently, grain sorghum is the most important cereal crop in Queensland, with grain yields reaching 2.73 tonnes/hectare (t/ha) [20]. In 2023, the average area of grain sorghum cultivation in Queensland and New South Wales was 452,000 ha and 170,000 ha, respectively [21]. There are very limited data on the agronomical characteristics of sweet sorghum. However, it is said to have similar growth features to that of grain sorghum. The average grain yield of sweet sorghum in Australia is 3–7 t/ha, with 115–120 days to attain physiological maturity [22]. In Queensland, sweet sorghum is predominantly cultivated as a sugarcane alternative and has been identified as a valuable feedstock for numerous biofuel products [23]. The sugars extracted from the sweet sorghum were used in bioethanol fermentation, while the lignocellulose residue bagasse was used as fodder or burnt in boilers to produce electricity [24,25].

An efficient way to utilise sweet sorghum as an energy crop is to cultivate it on sugarcane fields during the fallow period. Globally, research on sweet sorghum is often focused on optimising the fermentation of juice extracted from the stalk for bioethanol production [26]. In addition, the ensilation of sweet sorghum stalks is often practiced and used as feed for cattle, volatile fatty acid (VFA) production [27], and bacterial cellulose production [28]. These studies have established that sweet sorghum is a viable substrate for several forms of biofuel production. Its rapid growth and ability to reach maturity in 3 to 5 months makes sweet sorghum a favourable crop for cultivation on sugarcane fallow land. Moreover, sweet sorghum, which is grown during the rainy season, can be harvested and delivered to the sugar mill before the sugarcane harvesting season. The above factors also favour the off-estate cultivation of sweet sorghum by small-holder farmers [29]. Although there have been substantial studies on sweet sorghum use for biofuel production, there is little or no research on the use of sweet sorghum for commercial biogas production. The aim of this study was to cultivate the most popular sweet sorghum cultivars that can be grown during the fallow period of sugarcane cultivation in Queensland. The produced biomass will be used for biogas production. Further, the feasibility of utilising biogas for producing electricity or compressed biomethane (BioCNG) for each tested cultivar was also determined to evaluate the most suitable sweet sorghum cultivar for large-scale cultivation and biogas applications.

2. Materials and Methods

2.1. Experimental Site and Agronomic Practices

The experiment was carried out in 2020 at Arriga, Mareeba, Queensland (Latitude −17.014 and Longitude 145.306). Nine commercial sweet sorghum cultivars were sown in the fallow land after the harvest of sugarcane. Soil was a sandy loam with pH of 6.6 (1:5 water), cation exchange capacity of 3.72 cmol/kg, and electrical conductivity of 0.8 dS/m (1:5 water). Soil test report indicates organic carbon of 0.81% (Walkey-Black), 1.7 mg/kg of nitrate nitrogen, 1.5 mg/kg of ammonium nitrogen, 44 mg/kg of phosphorus (Colwell), 72 mg/kg phosphorus (BSES), and 0.05 cmol/kg of available potassium. During the sweet sorghum growing season (September to December 2020), the mean annual precipitation in Arriga was 81.5 mm, with an average temperature of 23.3 °C. The land was prepared by two passes of an offset disc harrow followed by a rotary hoeing. A bed former was used to construct raised beds of 20 m × 14 m × 1.8 m. Seeds were sown at 1.8 m row spacing. Prior to sowing (September), the beds were furrow irrigated. Upon drying out sufficiently, sweet sorghum seeds were sown at a seeding rate of 5 kg/ha at 0.8 m apart on each bed. For each cultivar, a plant density of 20,000 plants per ha was used.

Yaramilla complex fertilizer at a rate of 665 kg/ha was applied during sowing. This resulted in an application rate of 80 kg/ha of nitrogen, 33 kg/ha of phosphorous, 100 kg/ha of potassium, 100 kg/ha of magnesium, and 53 kg/ha of sulphur. Before sowing, the seed was treated with CONCEP II (active ingredient Oxabetrinil) at a rate of 1.8 g/kg of seed to protect it against chemical damage and enable the application of the pre-emergent herbicide. Pre-emergent herbicide Clincher (active ingredient: cyhalofop-butyl) was applied straight after sowing at a rate of 2 L/ha to control grass weeds. During the crop growth period, two irrigations of 100 mm were applied. Ten plants for each cultivar were randomly selected and earmarked for collecting biometric observations. Plant height, number of leaves, and fresh biomass were collected from these 10 plants. Harvesting was performed manually at 65 days after planting (DAP) using a cane knife. All samples were packed and shipped to the laboratory. At the time of harvest, the sweet sorghum stalks from a 5.56 m length of bed were harvested and weighed manually. This represents an area of 10 m^2. This fresh biomass weight was then multiplied by 1000 to get the biomass weight in t/ha.

2.2. Substrate and Inoculum

Table 1 presents the tested sweet sorghum cultivars and their chemical composition. In the laboratory, the plants were shredded to approximately a 1 cm particle size using a garden shredder (Ozito 2400 W). Samples were further oven-dried at 45 °C for 3–4 days. Dried samples were stored in hermetically sealed containers until further use.

Table 1. Chemical composition of sweet sorghum cultivars.

Substrate	TS (% *w*/*w*)	VS (% *w*/*w*)	Moisture (%)	VS/TS	C (%TS)	N (%TS)	C/N	TKN (gN/kgTS)	TKP (gP/kgTS)
Inoculum	2.98	2.07	97.02	0.70	34.54	6.25	5.53	55.91	26.87
SE-1	20.31	19.32	79.69	0.95	48.80	0.60	81.33	4.79	1.59
SE-5	15.24	14.29	84.76	0.94	48.70	0.70	69.57	5.99	1.53
SE-23	12.05	10.71	87.95	0.89	47.00	1.00	47.00	8.55	2.50
SE-35	14.25	13.11	85.75	0.92	47.80	1.10	43.45	6.06	1.86
SE-42	19.67	18.54	80.33	0.94	47.00	0.90	52.22	5.27	2.34
SE-45	18.26	17.24	81.74	0.94	47.70	1.10	43.36	6.92	1.52
SE-81	19.36	18.25	80.64	0.94	48.40	0.80	60.50	6.03	2.22
SE-86	19.61	18.54	80.39	0.95	47.30	0.80	59.13	5.75	1.78
Mega Sweet	21.40	19.83	78.60	0.93	47.50	1.00	47.50	7.11	2.35

Anaerobically digested material from a full-scale biogas plant treating sewage sludge and source-separated organic fractions of municipal waste (Luggage Point, Queensland

Urban Utilities, Brisbane, Australia) was used as inoculum. The collected inoculum was stored in a walk-in cold room (4 °C) until further use. Prior to the start of the experiments, the inoculum was degassed by incubating it at 37 °C for 5–7 days until the residual methane was minimal.

2.3. Biochemical Methane Potential Assays

Biochemical methane potential (BMP) of the tested substrates was measured in 160 mL glass serum bottles with a working volume of 100 mL. To each assay, 90 mL of inoculum and known number of substrates were added to achieve an inoculum to substrate ratio (ISR) of 2 on a volatile solid (VS) basis. Distilled water was added to achieve the desired working volume. The headspace of the assays was purged with 99.99% pure nitrogen (N_2) for a duration of 3 min. Following this, the assays were promptly sealed using butyl rubber stoppers and aluminium crimps. The assays were developed in triplicates and incubated statically at 37 °C in a thermostat-controlled incubator (Incucell). Inoculum-only assays were employed as a blank. Methane produced by the blank assays was subtracted from sample assays. The gauge pressure in the headspace was measured using a pressure transducer (Leo 2, Keller AG, Winterthur, Switzerland) in mbar and was converted to absolute pressure. The methane concentration in the assay was analysed using a gas chromatograph (Shimadzu 2014, Rydalmere, NSW, Australia) equipped with a thermal conductivity detector (TCD) according to a protocol described elsewhere [30].

2.4. Kinetic Modelling

The kinetics of methane production were measured using the first-order kinetic model and modified Gompertz model. These models were used to determine the lag phase and hydrolysis rate constant for the substrates. First-order kinetics is the simplest model used to predict the hydrolysis constant with the assumption that hydrolysis is the rate-limiting step [31]. This model helps to predict the time delay and methane yields during the anaerobic digestion process (Equation (1)). The time delay corresponds to the lag phase during which the bacteria try to acclimatize in the environment [32].

$$B(t) = B_0 * (1 - \exp(-kt)) \quad (1)$$

where B_0 = maximum specific methane production potential of the substrate (NmL CH_4/gVS_{added}); k_{hyd} is the hydrolysis rate constant (d^{-1}) and $B(t)$ = cumulative methane yield at digestion time t (d) (NmL CH_4/gVS_{added})

The Gompertz model was first used to identify the relationship between specific growth rate and bacterial population density [33]. However, it has been modified further with the assumption that the methane production rate in batch anaerobic digestion is proportional to the growth rate of methanogenic bacteria (Equation (2)).

$$B(t) = B_0 * \exp\left\{-\exp\left[\frac{R_{max} * e}{B_0} * (\lambda - t) + 1\right]\right\} \quad (2)$$

where $B(t)$ = cumulative methane yield at digestion time t (d) (NmL CH_4/gVS_{added}), B_0 is the maximum specific methane production potential of the substrate (NmL CH_4/gVS_{added}), Rmax = maximum methane production rate (NmL $CH_4/gVS_{\cdot added}$), λ = lag phase (day), t = time (day), and e = exp (1) = 2.7183.

2.5. Analysis

The analysis of the total solids (TSs) and volatile solids (VSs) was conducted in accordance with the Standard Methods [34]. Soluble sugars were extracted from starch, sucrose, and inulin using a mild acid hydrolysis procedure and measured using the anthrone method. [35]. According to a methodology previously reported, structural carbohydrates (glucose, xylose, and arabinose) and uronic acids, such as galacturonic and glucuronic acids

derived from cellulose, hemicelluloses, and pectins, were quantified in triplicates using a strong acid hydrolysis [36].

Cellulose and hemicelluloses contents were estimated as shown below:

$$\text{Cellulose (\%TS)} = \text{Glucose (\%TS)}/1.11$$

$$\text{Hemicelluloses (\%TS)} = [\text{Xylose (\%TS)} + \text{Arabinose (\%TS)}]/1.13$$

where 1.11 is the conversion factor for glucose-based polymers (glucose) to monomers, and 1.13 is the conversion factor for xylose-based polymers (arabinose and xylose) to monomers.

Total soluble sugar content was estimated as shown below [37]:

$$Y = 0.8111x - 0.37285$$

where y = total soluble sugar content, %; x = Brix of stalk juice, °Bx.

Proteins were determined by multiplying total Kjeldhal nitrogen (TKN) by 6.25 [38]. TKN was analysed using a Buchi digestion unit K438 and a Buchi 370-K distillator/titrator [39]. The composition of volatile fatty acids (VFAs) in the liquid phase, specifically acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and iso-valeric (C5 and iC5), and caproic (C6) acids, was analysed by using a gas chromatograph equipped with a flame ionization detector (FID) [40]. The determination of the total phenols in the liquid fractions was conducted by using a microtube test (Spectroquant®, Merck, Darmstadt, Germany) accompanied by a colorimetric measurement using 4-aminoantipyrine after a dilution of 1:200 [41].

Gas volume in the assays was calculated by using Equation (3), where headspace is assumed as initial volume (V1). The gas production potential (defined as CH_4 potential) of the substrates were given as the amount of CH_4 produced in NmL CH_4/gVS_{added}.

$$BMP_t = \frac{ml\ CH_4\ sample\ assay - ml\ CH_4\ inoculum\ assay}{Volatile\ solids_{substrate}} \quad (3)$$

where mL *CH4 sample assay—mL CH4 inoculum assay* is the net methane volume (mL) obtained from the one substrate only, adjusted to the standard temperature (0 °C) and pressure (1 atm) condition (STP); VS_{added} is the mass of substrate VS in the sample bottle (g). The methane production per hectare can be determined by multiplying the specific methane yield (measured in NmL CH_4/gVS_{added} by the biomass yield.

2.6. Statistical Analyses

The results of the chemical analysis and biomethane potential tests are presented as the mean values and the standard errors of the mean of the three replicates used in the study. The effects of chemical composition and methane potential of the nine cultivars were evaluated for significance using IBM SPSS Statistics® software (https://www.ibm.com/spss) and one-way analysis of variance (ANOVA) or *t*-test with Tukey's post hoc test at a significance level of 0.05.

3. Results and Discussions

3.1. Chemical Composition of Sweet Sorghum Cultivars

The chemical composition of sweet sorghum cultivars is presented in Table 1. The results showed that the TS content in the nine sweet sorghum cultivars ranged between 12.05% (SE-23) and 21.40% *w/w* (Mega Sweet). A similar trend was also noticed with VS. The high VS/TS of 0.89–0.95 indicates that all the tested cultivars were rich in organic matter and are considered as ideal substrates for biogas production. This result agrees with previous studies in which substrates with high VS/TS were rich in organic matter [42]. The carbon content in sweet sorghum cultivars was more or less similar (Table 1). SE-1 had the highest carbon content of 48.8 %TS, while SE-42 and SE-23 had the lowest carbon content of 47.0 %TS. On the other hand, the nitrogen content in the tested cultivars was in the range of 0.6 to 1.10 %TS. Thus, the carbon to nitrogen (C/N) ratio ranged from a low

value of 43.33 (SE-45) to a high value of 81.36 (SE-1). However, the optimum C/N ratio for anaerobic digestion (AD) is 20–30:1 and is considered to provide sufficient nitrogen to promote the growth of microbes and boost the degradation of organic carbon [43]. At a high C/N ratio, methanogens will rapidly utilise the nitrogen to meet their protein needs and then not utilize the remaining carbon. Consequently, gas production will be decreased [44]. Nonetheless, high VS/TS and C/N ratios indicate that the substrates are rich in carbon that may be difficult to degrade during the AD process due to the presence of lignin, and hydrolysis might be the rate-limiting step for these substrates. The concentrations of TKN and TKP in the substrates are presented in Table 2. The TKN values ranged from 4.79 gN/kgTS in SE-1 to 7.11 gN/kgTS in the Mega Sweet cultivar. Conversely, SE-23 had the highest TKP concentration (2.50 gN/kgTS).

Table 2. Plant height, harvest time (days after planting, DAP), and biomass yields along with total soluble and insoluble sugar content in the nine sweet sorghum cultivars.

Substrate	Plant Height (m)	Number of Leaves	Harvest Time (DAP)	Fresh Biomass (t/ha)	Total Soluble Sugars	Cellulose (%TS)	Xylan (%TS)	Arabinan (%TS)	Galactan (%TS)	Lignin (%TS)	Ash (%TS)	Total Soluble Sugars (t/ha)	Total Insoluble Sugars (t/ha)	Total Sugars (t/ha)
SE-1	1.86	11	65	67.0	12.3	33.2	18.2	1.6	2.4	19.2	1.5	8.24	37.12	45.36
SE-5	2.8	14	65	57.4	1.4	31.8	18.4	1.5	2.4	24.5	2.7	0.80	31.05	31.86
SE-23	1.96	12	65	78.4	1.4	35.5	19.3	1.1	1.7	22.3	1.4	1.10	45.16	46.26
SE-35	2.56	12	65	82.3	3.1	30.3	17	0	2.1	24.4	2.7	2.55	40.66	43.21
SE-42	3.93	14	65	67	2.1	37.5	21.1	1.4	2.3	25.1	1.4	1.41	41.74	43.15
SE-45	3.58	14	65	58.4	11.9	28.1	16.4	1.2	2.1	23.2	1.2	6.95	27.92	34.86
SE-81	3.12	14	65	77.5	9.2	25.8	14.4	1	1.8	23.2	2.7	7.13	33.33	40.46
SE-86	3.26	13	65	70.8	13.5	27.5	15.6	1.1	2	22.8	1.6	9.56	32.71	42.27
Mega Sweet	2.88	13	65	46.9	9.6	26.9	15.3	1.3	1.9	24.1	0.4	4.50	21.29	25.80

The total soluble sugars, insoluble sugars (carbohydrate), and lignin content in the studied cultivars are presented in Table 2. The total soluble sugar content varied from cultivar to cultivar and was shown to be dependent on the plant height and duration. Given that plant height depends on the maturity of the crop and internode length, there is a strong correlation between plant height and crop biomass yield [45,46]. It has been observed that if a sweet sorghum plant is producing more leaves and nodes, then it is said to be in an extended vegetative stage. As a result, late-flowering cultivars are typically taller than early-flowering cultivars. However, dwarf genes govern the plant height in hybrids, and they achieve the same number of leaves and leaf area by lowering the internode length [47].

The higher total soluble sugar content in the SE-86 cultivars than in SE-5 or SE-23 cultivars is attributed to the fact that sugar accumulation generally takes place in stems, and thus tall plants tend to accumulate more total soluble sugars (Table 2). In the present study, the SE-86 cultivar was 3.26 m tall, which is taller compared to the SE-5 (2.8 m) or SE-23 (1.96 m) cultivars. These results agree with previous studies where the sucrose concentration in sweet sorghum was shown to increase rapidly after flowering [13] and was shown to correlate with carbohydrate metabolism enzymes and the onset and extent of sugar accumulation in both sugarcane (Saccharum spp.) and sweet and grain (non-sweet) sorghums [48–50]. However, a decline in sucrose-degrading activity was shown to be a prerequisite for sugar accumulation [51]. Interestingly, no apparent difference in the activities of specific enzymes and higher stem sugar concentration was reported in sweet vs. grain sorghum cultivars [52].

The cellulose content in the nine tested sweet sorghum cultivars ranged between 25.80 and 37.50 %TS (Table 2). The SE-42 cultivar had the highest cellulose content (37 %TS), while the lowest cellulose content was seen in the SE-81 cultivar (25.81 %TS). A similar trend was also noticed with the hemicellulose and lignin contents (Table 2). However, no significant difference in the lignin composition across the tested cultivars was seen, suggesting that the cellulose content did not increase proportionately with the increase in lignin content. Both of these results suggest that the biomass yields were directly related to the total insoluble sugars, especially with the carbohydrate and lignin compositions in a cultivar (Table 2). In sweet sorghum, carbohydrates are generally accumulated and stored in the stems. This limits the amount of carbohydrates available for storage in the grain [53].

The opposite is true for grain sorghum, in which most of the carbohydrates are diverted and stored in the grain [47].

Figure 1 presents the relationship between plant height, plant biomass, number of leaves, and lignin content and the total sugar and methane yields. The Bubble plots show that there is a positive correlation between the plant height and fresh biomass and the total sugar content and thus the methane yields. Cultivars SE-23, SE-35, SE-81 and SE-86 had >70.8 t/ha of fresh biomass. The corresponding total sugar content was 42–46 t/ha. The methane yields for the above four cultivars were between 21.2 and 39.5 m^3/tFM. The lower methane yields observed for the SE-23 and SE-35 cultivars than for the SE-81 and SE-86 cultivars were obviously due to lower VS content in the former than the latter sweet sorghum cultivars.

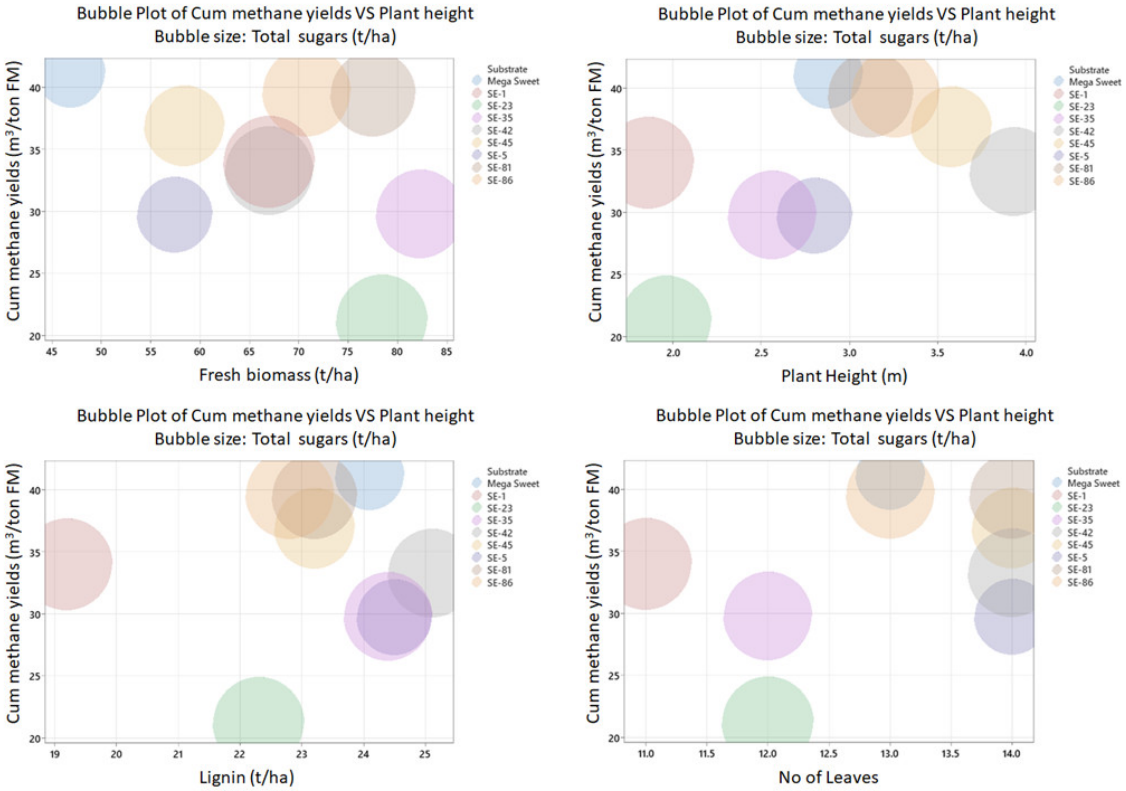

Figure 1. Bubble plots showing the relationship between the total sugar content and methane yields with respect to plant biomass, plant height, lignin content, and number of leaves.

3.2. Biochemical Methane Potential Yields

The methane production rates and yields for all cultivars at an ISR of 2 are presented in Figure 2 and Table 3. Methane production started immediately in all assays, and higher methane production rates and yields were noticed in all cultivars except for SE-1 (Figure 2). The cumulative methane yields after 34 days of incubation ranged from 175 to 227 NmLCH_4/gVS_{added} for the tested cultivars. The methane yields obtained in the present study were comparable with the methane yields reported in the literature [54–58]. For instance, Mahmood et al. [56] tested 14 sorghum cultivars and reported methane yields of 250–354 NmL CH_4/gVS_{added}. However, the methane yields per ha in the above study (3924 to 7120 Nm^3 per ha) were higher than in the present study, which ranged from 1664 to 3059. This discrepancy is attributed to the fact that cultivars with high biomass yields per ha and methane yields resulted in an overall high methane yield per hectare, and vice versa. Similarly, Sambusiti et al. [55] reported methane yields ranging from 270 to 335 NmL CH_4/gVS_{added} for five untreated sweet sorghum hybrids and observed that alkaline pretreatment did not improve the methane yields (288–336 NmL CH_4/gVS_{added}). The above result suggests that the effect of sodium hydroxide pretreatment on

the methane yields is dependent on the composition and type of substrate employed. Finally, the methane yields reported in this study were also within a ranges of 232–316 NL CH_4/kg VS_{added} [56], 216–316 NmL CH_4/gVS_{added} [58], and 338 NmL CH_4/gVS_{added} [54] reported for sweet sorghum cultivated at higher latitudes and temperate climates. The results thus suggest that the methane yields from sorghum are not much influenced by the extent of its cultivation but by the degradability of biomass and its chemical composition.

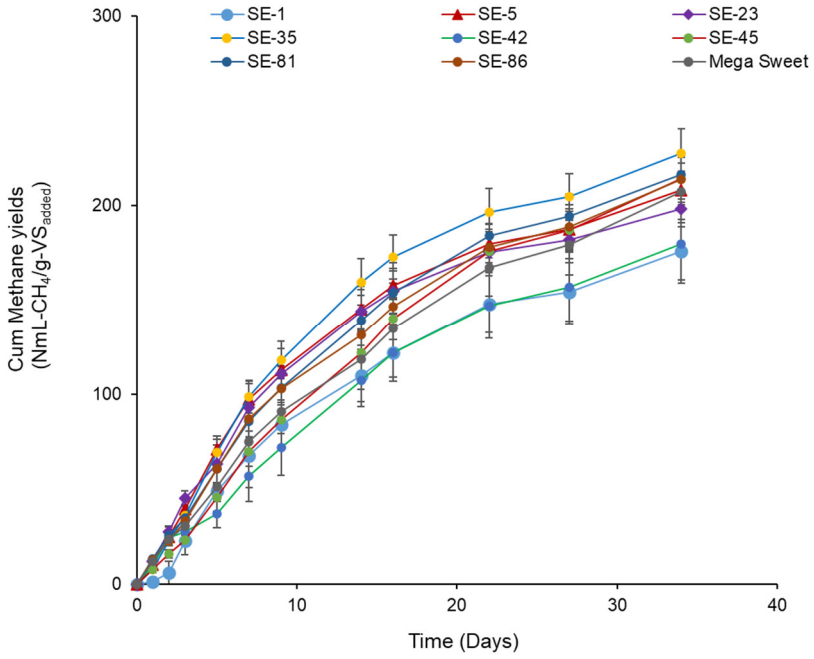

Figure 2. Methane production rates and cumulative methane yields of the studied nine sweet sorghum cultivars incubated in batch at ISR 2 and 37 °C.

Table 3. Cumulative methane yields and VS removal rates obtained during the anaerobic digestion of sweet sorghum cultivars in batch experiment at ISR 2 and 37 °C.

Cultivar	Cum Methane Yields (NmL CH_4/gVS_{added})	Cum Methane Yields (m^3/t FM)	VS Removal (%)
SE-1	175.93 ± 15.10	33.98	69.22
SE-5	208.05 ± 9.81	29.73	70.69
SE-23	198.37 ± 9.50	21.24	62.01
SE-35	227.48 ± 12.86	29.83	69.26
SE-42	179.66 ± 20.84	33.32	76.98
SE-45	214.30 ± 11.08	36.94	72.06
SE-81	216.31 ± 11.35	39.47	78.74
SE-86	213.75 ± 12.20	39.63	80.83
Mega Sweet	207.47 ± 14.89	41.15	63.59
Sewage Sludge	92.90 ± 0.80	1.93	13.03

The relatively high methane yields obtained for the SE-35 cultivar were attributed to its high C/N ratio of 43.5 (Table 1). According to the literature, the ideal C/N ratios for methane generation were determined to be within a wide range of 10–40, with 20–30:1 being the optimal C/N ratio under wet anaerobic digestion [59]. Conversely, the low methane yields noticed for the SE-1 cultivar suggest that its high C/N ratio of 81.33 may have led to the build-up of organic acids and a lack of sufficient inoculum to process the intermediate metabolites [60,61]. Thus, this limitation can be overcome by performing the AD process at a higher ISR of 4 or by co-digestion with nitrogen-rich substrates such as animal manure or food wastes that can adjust the C/N ratio optimally for the AD process [23,62]. On

the contrary, the Mega Sweet cultivar produced the highest methane yields of 41.15 m^3/t compared to cultivars with higher cellulose content (Table 2). These results are in accordance with previous results, where the lignocellulosic biomass with the lowest cellulose and higher lignin content had lower biogas yields [63]. On the other hand, the low methane yields for the SE-1 cultivar, despite having high cellulose content (>30 %TS) and low lignin content (19.20 %TS), suggest that the AD process efficiency cannot be calculated solely based on the carbohydrate composition, as it is also influenced by other physiochemical factors such as the type of carbon and its biodegradability, as well as AD process conditions.

3.3. Chemical Composition of Digestate

The chemical composition of the digestates upon the termination of the experiments is presented in Tables 4 and 5. The pH in all digestates was 7.4–7.5. The inhibition due to ammonia was insignificant, as the ammonia concentration in all assays was <3000 mg/L [64]. Similarly, the total VFA composition (29.59 to 73.68 mg/L) was also lower than the threshold level for inhibition due to VFAs [65]. Acetic acid was the major VFA component in all assays, accounting for 47.22–76.71% of the total VFA. The highest total VFA composition of 73.68 mg/L seen in the SE-1 cultivar also resulted in the lowest methane yields (175.13 $NmLCH_4/gVS_{added}$), suggesting VFA accumulation [66].

Table 4. Total volatile fatty acids (VFAs) and individual VFA (mg/L) component analysis after termination.

Cultivar	SE-1	SE-5	SE-23	SE-35	SE-42	SE-45	SE-81	SE-86	Mega Sweet	Inoculum	Cellulose
Total VFA	73.68	48.48	29.59	41.92	42.71	48.68	48.97	59.39	51.24	53.09	55.16
Acetic acid	34.79	35.28	22.7	28.84	29.04	29.09	31.01	35.2	32.63	29.92	32.36
Propionic acid	4.58	4.75	4.04	4.86	4.26	6.76	6.6	10.46	7.09	6.09	7.13
iso-Butyric acid	1.49	1.75	1.47	1.56	1.4	2.63	2.32	2.55	2.55	2.41	2.34
Butyric acid	23.9	1.69	1.38	1.62	1.33	2.44	3.05	2.11	1.96	2.21	2.01
iso-Valeric acid	2.65	2.17	0	2.2	2.48	3.91	2.87	3.32	4.08	3.62	3.29
Valeric acid	4.43	2.85	0	2.84	4.21	3.86	3.12	3.46	2.94	3.54	3.62
4-Methyl valeric acid	1.84	0	0	0	0	0	0	0	0	3.25	3.19
Hexanoic acid	0	0	0	0	0	0	0	2.3	0	2.05	1.22

The major nutrients required to cultivate sweet sorghum are N, phosphorus (P), potassium (K), and sulphur (S) [67]. Among the cultivars, the digestate from the Mega Sweet assay had the highest N, P, and S contents, with a N:P:K:S ratio of 1:0.3:9.8:0.6 (Table 5). A recent study reported that sweet sorghum requires 190 kg/ha of N, 28 kg/ha of P, and 0.375 kg/ha of zinc (Zn) [68]. The fertiliser requirements may vary depending on the area of cultivation and soil properties. Although the direct application of a digestate may not solve the fertilizer requirement for sweet sorghum cultivation, there is scope for additional digestate treatment to enhance the nutrient recovery for fertilizer use [69]. A solid–liquid separation or a vacuum evaporation process can be used further downstream to enhance the digestate composition for fertilizer application [70]. Likewise, the digestate can also be substituted with inorganic fertilizers, which is much cheaper compared to the application of only inorganic fertilizers alone for sweet sorghum cultivation [71].

3.4. Kinetics of Methane Production during the Anaerobic Digestion of Sweet Sorghum Cultivars

Table 6 provides a summary of the kinetic parameters obtained from both models. The experimental data associated with methane production were compared with the corresponding parameters B_o and G_o derived from the first-order and modified Gompertz equations, respectively (Table 6). The results indicate that the first-order model provided the most accurate fit to the experimental data, as there was no observable lag phase in the investigation. The first-order model does not account for a lag phase and characterizes the AD process only after the exponential phase of methane production has begun. However, when the process needs longer adaptation periods, the modified Gompertz equations fit

better, which was evident from the higher determination coefficients (R^2) [72]. Among the two studied models, the first-order model showed a lower difference between the experimental and predicted methane yields (0.11 to 2.80%) than the modified Gompertz model (3.67 to 7.21%). In addition, to assess the soundness of the model, the rRMSE and R^2 values were determined. A lower rRMSE and higher R^2 values were obtained for the first-order model than modified Gompertz model [73].

Table 5. Major- and micro-nutrient composition (mg/kg FM) of the digestate at the end of the batch experiment.

Cultivar/Nutrients	SE-1	SE-5	SE-23	SE-35	SE-42	SE-45	SE-81	SE-86	Mega Sweet
Al	7.57	17.44	15.05	9.75	9.02	3.81	18.06	5.92	14.57
As	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ca	488.43	472.85	535.37	507.27	720.42	533.53	545.51	459.01	871.98
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Co	0.38	0.29	0.23	0.00	0.34	0.31	0.00	0.35	0.42
Cr	3.08	2.49	2.25	1.54	2.08	1.37	1.94	2.06	1.96
Cu	0.97	0.74	0.68	0.81	1.06	0.85	0.88	0.83	1.64
Fe	18.72	19.62	18.55	12.59	15.88	9.25	17.89	12.42	17.69
Hg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K	2920.51	2256.72	3666.05	3468.23	3413.90	2943.51	2414.42	2720.89	3177.04
Mg	313.93	303.69	291.07	254.09	355.74	314.20	368.50	261.92	366.46
Mn	7.87	4.48	4.16	5.83	4.96	3.25	4.50	3.45	9.39
Mo	0.00	0.00	0.22	0.26	0.00	0.00	0.32	0.00	0.00
Na	18.57	12.10	9.66	7.58	23.33	12.17	22.79	13.01	13.92
P	290.85	248.72	298.31	273.48	441.35	361.08	364.95	300.91	504.35
Pb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	141.64	107.40	115.04	110.85	175.14	128.36	138.99	120.52	202.29
Si	10.43	0	5.15	6.11	0	0	7.26	7.46	9.66
Se	0.90	0.00	0.00	0.77	0.00	0.00	0.00	0.00	1.39
Zn	4.09	2.88	2.29	2.63	3.90	2.86	3.27	2.97	4.17
TKP	65.57	35.54	36.31	37.78	90.50	50.67	83.19	68.45	107.64
TKN	197.55	139.15	124.18	123.08	203.82	230.69	225.97	221.13	325.66
N:P:K:S	1:0.3:14.8:0.7	1:0.3:16.2:0.8	1:0.3:29.5:0.9	1:0.3:28.2:0.9	1:0.4:16.7:0.9	1:0.2:12.8:0.6	1:0.4:10.7:0.6	1:0.3:12.3:0.5	1:0.3:9.8:0.6

Table 6. First-order and modified Gompertz modelling analysis for all cultivars.

Substrate	Exp BMP	First order Kinetics						Modified Gompertz							
		B_0	t-delay	k_{hyd}	R^2	RMSE %	% Difference	Rmax	λ	G_0	R^2	RMSE %	% Difference	T_{90}	T_{eff}
Units	(NmL CH_4/g-VS_{added})	(NmL CH_4/gVS_{added})	(d)	(d^{-1})		(%)	(%)	(NmL CH_4/gVS_{added} d^{-1})	(d^{-1})	(NmLCH_4/gVS_{added})		(%)	(%)	(d)	(d)
SE-1	175.93	188.61	1.22	0.07	0.997	4.09	2.80	9.61	0.93	166.62	0.983	10.17	6.96	28.30	27.37
SE-5	208.05	214.27	0.46	0.09	0.998	2.90	2.74	12.69	0.10	194.88	0.986	7.96	7.21	26.99	26.89
SE-23	198.37	206.90	0.32	0.09	0.999	2.44	1.35	12.29	0.00	188.81	0.990	6.59	5.60	24.32	24.32
SE-35	227.48	238.40	0.75	0.08	0.999	2.69	1.95	13.93	0.58	214.51	0.990	7.01	6.61	27.05	26.46
SE-42	179.66	236.25	0.07	0.04	0.996	4.92	0.36	8.16	0.42	181.05	0.994	5.97	3.67	28.49	28.07
SE-45	214.30	273.84	0.71	0.05	0.998	3.32	0.11	10.03	1.00	214.02	0.993	6.87	4.19	28.54	27.53
SE-81	216.31	245.53	0.24	0.06	0.999	2.06	0.61	11.08	0.08	210.83	0.991	6.73	4.71	27.05	26.97
SE-86	213.75	242.33	0.20	0.06	0.998	3.46	1.72	10.57	0.00	206.51	0.986	8.57	5.84	28.03	28.03
Mega Sweet	207.47	255.18	0.00	0.05	0.998	3.12	1.83	9.18	0.00	206.28	0.988	8.15	5.01	28.86	28.86

Note: Expt BMP: experimental biochemical methane yields (NmLCH_4/g-VS_{added}); Rmax = maximum methane production rate (NmLCH_4/g-VS_{added} d); G_0: maximum cumulative methane yield (NmLCH_4/g-VS_{added}); λ = lag phase (d); B_0: maximum cum methane yield (NmLCH_4/g-VS_{added}); k_{hyd} (d^{-1}): hydrolysis constant; T-delay: time delay (d).

It can be observed that the highest R_{max} value was obtained for the SE-35 cultivar, followed by the SE-23 and SE-5 cultivars. The value of λ determines the minimum time required for biogas production [74]. Despite the higher biogas production rate, the time constant for the SE-35 cultivar, according to modified Gompertz model, was higher than the substrates producing lower biogas yields, such as Mega Sweet. This could be possible due to the readily available sugars in the Mega Sweet cultivar that are easily degraded to produce biogas, resulting in a faster hydrolysis rate.

Table 6 also shows the effective methane production period (T_{ef}). T_{ef} was calculated by subtracting the lag time (k) from T_{90}. The time needed for 90% methane production (T_{90}) fell within the range of 23.32–28.86 days. The T_{ef} values ranged from 24.32 to 28.86 days. Overall, the Mega Sweet cultivar had the highest T_{ef} values followed by the SE-42 and SE-86 cultivars. The high T_{ef} values in these samples may be the result of

rapid acidification, which inhibits methanogenesis. This was evident from the low R_{max} values for the Mega Sweet cultivar, which had a high T_{ef} (28.86 days) and a low R_{max} (9.18 L $CH_4/kgVS_{added}/day$) and produced relatively high methane yields of 207.47 NmL $CH_4/kgVS_{added}$. The observed differences in the rate of methane production may be attributed to the presence of a readily degradable substrate, slowly degradable substrate, and the quantity and nature of the intermediate products formed during the anaerobic digestion process.

3.5. Correlation of Chemical Composition and Methane Yields Using Principal Component Analysis

The interaction of active variables (parameters) such as TS, VS, plant height, fresh biomass, total soluble sugars, cellulose, hemicellulose, xylan, arabinan, galactan, ash, hydrolysis constant (k), and moisture content on the methane yields were subjected to a principal component analysis (PCA). The results of the PCA, including the active observations (cultivars) and active variables (parameters), are presented in Figure 3. As only the first two components were meaningful (eigenvalue > 1), only two principal components are presented. Nine sweet sorghum genotypes (active observations) were grown under similar environmental conditions and were harvested at the same time. Overall, four clear groups of cultivars were identified. The Mega Sweet, SE-1, and SE-42 cultivars are clustered on the top right part of the plot, with a higher-than-average content of holocellulose (hemicellulose and cellulose) variables. On the other hand, the SE-35 cultivar was at the extreme lower left of the plot with a high ratio of fresh biomass and ash content. The SE-81, SE-86 and SE-45 cultivars were located at the extreme right with relatively high plant height, total soluble sugars, TS, VS, and lignin contents than average and were positively influenced by methane yields. Cultivars SE-23 and SE-5 were located on the top left with a higher moisture content and hydrolysis constant, k, than average and were less influenced by methane yields.

Among the cultivars, SE-35 was strongly influenced by fresh biomass and ash, which is a non-digestible variable. On the other hand, cultivars SE-45, SE-81, SE-86 were also influenced by these variables but to a lesser extent than the SE-35 cultivar. The Mega Sweet, SE-1, and SE-42 cultivars were also positioned closer to lignin, TS, and VS and further away from fresh biomass. The results thus suggest that plant height, total soluble sugars, lignin, TS, and VS had greater impacts on sweet sorghum cultivar screening. The interesting point revealed in this study was that methane yield is highly correlated with lignin content, even more than biomass yield. Therefore, applying the pretreatments, which are aimed at lignin removal or solubilisation, would be appropriate for biogas production. While the chemical composition of sweet sorghum in Table 1 shows high VS/TS and C/N ratios, the VS removals indicate that most of the carbon was undegraded during the AD process (Table 3). Thus, an additional pretreatment process may be necessary to further improve the biodegradability and methane yields of the studied sweet sorghum cultivars [55]. Moreover, by adjusting the C/N ratio, co-digestion with nitrogen-rich substrates such as livestock manure may further improve methane yields.

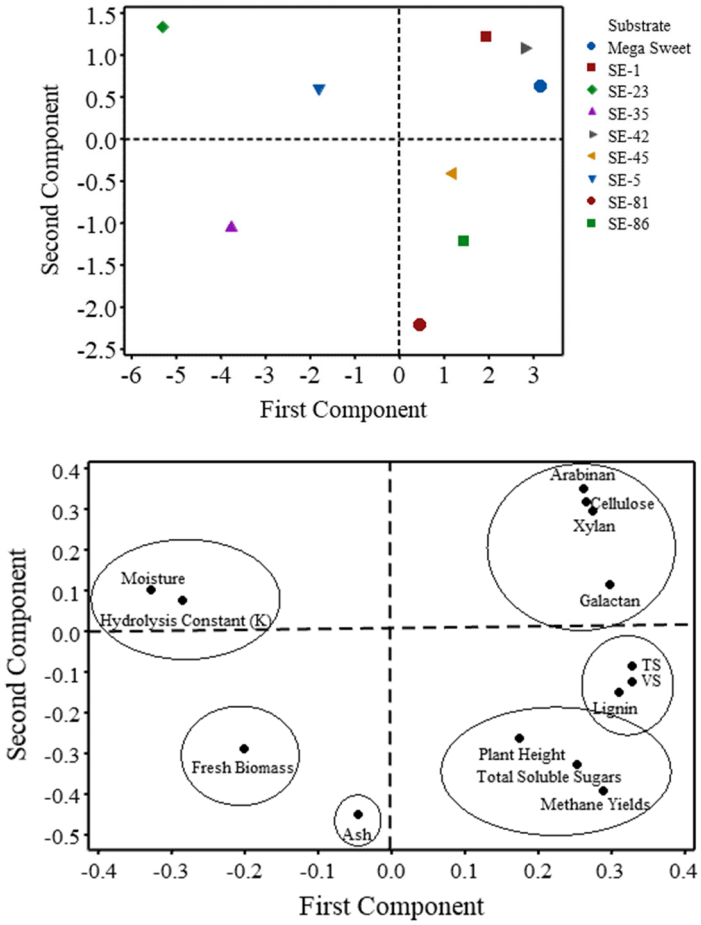

Figure 3. The plot of the loading map (above) and score map (below) obtained from a principal component analysis (PCA) of methane production from nine different Sweet Sorghum cultivars.

3.6. Energy Production Calculations

Table 7 presents the total gross energy production in biogas and its utilization for heat and electricity generation in a combined heat and power (CHP) plant or upgraded to biomethane and compressed for vehicle fuel use (bioCNG). In this study, of the total 226 ha of sugarcane cultivation, 25 ha have been designated as fallow land for the cultivation of sweet sorghum. Based on the total biomass production and methane yields obtained in the present study, the gross energy production was calculated. The estimated biomass yields for the tested sweet sorghum cultivars ranged from 46.90 to 82.30 t/ha/a. In addition to the high biogas yields, the net energy yield is also an essential parameter in selecting a suitable cultivar. As shown in Table 7, a significant difference was noticed between the methane yields and methane yields per ha across the tested sweet sorghum cultivars. The calculated gross energy for the tested cultivars was between 414.56 and 761.74 MWh/a. Interestingly, the SE-35 cultivar, with highest biomass yields and highest methane yields, had lower methane yields (m^3/ha) than the values obtained for the SE-81 and SE-42 cultivar. Based on the biomass yields and methane production, the highest methane yields of 3059.18 m^3/ha were obtained for the SE-81 cultivar, with a corresponding gross energy potential of 761.74 MWh/a. The produced energy could be used to produce 322.98 kW_{el}/ha/a of electricity and 324.50 kW_{th}/ha/a of heat in a CHP with an 85% conversion efficiency. Alternatively, the use of the total biogas for bioCNG production could generate 74,949.85 Nm^3/ha/a of vehicle fuel. The produced bioCNG could fuel 94.6 passenger cars per year (@10,000 km/a). Nonetheless, the surplus heat energy could also be used for the pretreatment of sweet sorghum biomass to further improve the methane yields per hectare or to fulfill the energy requirements of the biogas plant [75]. In addition, the use of digestate, that is rich in nutrients and carbon, a by-product of the AD process,

allows for the inorganic fertilizers to be substituted and can remediate the damaged soils. Further, a digestate containing mainly lignin and other undegraded carbohydrates can be separated into solids and utilised in boilers at sugar mills as an additional fuel source [76]. Substituting inorganic fertilisers with digestate can also avoid the GHG emissions associated with their production and application. However, the use of fallow land for cultivating the selected sweet sorghum cultivars as an energy crop is considered an unsustainable farming practice due to the land-use competition between food/fodder and energy production. Therefore, the economic and environmental benefits for the farmers in terms of extracting energy through the AD of sweet sorghum must be carefully evaluated using a systems approach. As stated in the literature, sweet sorghum requires limited nutrient and water content, so there is no net loss in soil quality for the next sugarcane cultivation. Regional plans on biomass and land use allocation plans should be developed for the mid- to long-term.

Table 7. A comparison on total gross energy, heat, and electricity generation in a combined heat and power (CHP) plant or compressed biomethane (BioCNG) production for vehicle fuel for the nine tested sweet sorghum varieties.

Parameter	SE-1	SE-5	SE-23	SE-35	SE-42	SE-45	SE-81	SE-86	Mega Sweet
Plant Population (per ha)	151,151	130,104	172,197	135,844	130,104	107,145	166,457	170,284	116,711
Fresh biomass (t/ha)	67.00	57.40	78.40	82.30	67.00	58.40	77.50	70.80	46.90
Cum methane production (NmL CH_4/gVS_{added})	175.93	208.05	198.37	227.48	179.66	214.30	216.31	213.75	207.47
Methane production (Nm^3/t FM)	33.98	29.73	21.24	29.83	33.32	36.94	39.47	39.63	41.15
Biomethane (Nm^3/ha)	2276.86	1706.67	1664.88	2454.84	2232.28	2157.34	3059.18	2805.89	1929.73
Biomethane (m^3)	56,921.59	42,666.84	41,622.11	61,370.98	55,806.99	53,933.58	76,479.44	70,147.21	48,243.26
Gross energy potential (MWh/ha/a)	566.94	424.96	414.56	611.25	555.84	537.18	761.74	698.67	480.50
CHP-Electrical production (kW_{el}/ha/a)	240.38	180.18	175.77	259.17	235.68	227.76	322.98	296.23	203.73
CHP-Heat production (kWh_{th}/ha/a)	241.52	181.03	176.60	260.39	236.79	228.84	324.50	297.63	204.69
BioCNG production (Nm^3/ha/a)	55,783.16	41,813.51	40,789.67	60,143.56	54,690.85	52,854.91	74,949.85	68,744.27	47,278.40
Fuel for passenger cars	70.67	52.97	51.68	76.20	69.29	66.96	94.96	87.09	59.90

Note: 1 m^3/t methane produces 9.66 kWh energy; CHP unit: 85% of gross energy potential (heat efficiency—42%; electricity efficiency—42%); BioCNG—98% methane content; 1 m^3 bioCNG = 12.67 km distance travelled, assuming passenger car travels 10,000 km/a.

4. Conclusions

The present study demonstrated that sweet sorghum can be grown during the fallow period of sugarcane cultivation. The results showed that the methane yields of the nine sweet sorghum cultivars tested were dependent on their chemical composition and biomass yields. Methane yields of 21.24 to 41.13 m^3/t FM were obtained for the nine cultivars tested. Cultivars with high total sugar contents and plant biomass resulted in high methane yields (SE-81 and 86). On the other hand, the SE-35 cultivar had the highest biomass (82.3 t/ha) and experimental methane yield of 227.48 NmL CH_4/gVS_{added} but resulted in a low per ha methane yield of 29.83 m^3/tFM. Thus, the SE-81 cultivar was selected as a promising sweet sorghum cultivar due its high methane yield (3059.18 m^3/ha) and gross energy yield. The estimated gross energy per ha of the SE-81 cultivar was 29.55 MWh/ha, or fuel for 37.98 passenger cars per hectare per annum. An integrated biorefinery using sweet sorghum as an energy crop for biogas production is a promising solution for sugarcane farmers. However, the effect of different pre-treatments, such as hydrothermal, alkaline, and/or steam explosion of sweet sorghum should be investigated to improve the hydrolysis and methane yields. Further, the co-digestion of sweet sorghum with animal manure also needs to be investigated, as the C/N ratio was below the optimal ratio during the mono-digestion of sweet sorghum. Finally, a comprehensive techno-economic feasibility study is needed for a sweet sorghum-based biorefinery to assess the environmental and economic

benefits of using sweet sorghum as an energy crop grown on the fallow lands under sugarcane cultivation.

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