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Ensiling parameters in vertical columns and multiple kinetic models evaluation of biomethane potential of ensiled sugar beet leaves

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Abstract: The quality of silages could deteriorate during feed-out to biogas reactors. Using airtight silos where silages in pulp form can be pumped directly into a reactor may mitigate this problem. In this study, sugar beet leaves were ensiled in vertical columns and in airtight bags at ambient temperature for 370 days. Homofermentative lactic acid bacteria were added to some of the samples in the airtight bags to test the effect on silage quality and biomethane potential (BMP). Quality of ensiling and BMP were studied across the height of the columns. With the exception of the silages at the top of the columns, lactic acid represented over 55% of the concentration of total fermentation products in the silages. The silages at the bottom of the columns had a 20.9 % higher BMP than the silages at the top, indicating that the BMP increases with the column depth. The BMP of the silage with additive in the airtight bags was 8 % higher than that of the silage without additive. Four kinetic models were used to fit the experimental BMP, out of which the one-step two-fraction kinetic model described the experimental BMP better than other models.

Keywords: vertical columns; lactic acid; kinetics; airtight bags

Introduction

Sugar beet leaves (SBL) are a by-product of sugar beet production with a relatively low total solids (TS) content between 13.2 to 14.2 % of fresh mass (FM) and volatile solids (VS) contents between 10.60 to 11.65 % of FM (Kreuger et al. 2011; Aramrueang et al. 2017; Larsen et al. 2017). Due to the seasonal harvest and in order to ensure that SBL are available as substrate for biogas production whenever required, they have to be preserved. One effective and cheap method for the preservation of wet biomasses such as SBL is ensiling.

The process of ensiling involves the conversion of water soluble carbohydrates (WSC) into organic acids such as lactic and acetic acids (Franco et al. 2018). Lactic acid reduces the pH of the system during ensiling, thereby preventing the silage from deteriorating before being fed to reactors. Acetic acid is responsible for the stability of a silage when it is exposed to air (aerobic stability) during the feed-out stage (Wilkinson and Davies 2013). Another important organic acid that can be produced during ensiling is butyric acid. This acid is produced by clostridia through the fermentation of sugars or lactic acid. The formation of butyric acid results in increased pH, energy loss and loss of TS in the system (Muck 2011). The major alcohol present in silages is ethanol. A high concentration of ethanol is an indication of yeast fermentation and usually leads to VS losses (Kung et al. 2018).

Ensiling has also been reported to be a pre-treatment process (Teixeira Franco et al. 2020), especially when enzymes that can convert complex carbohydrates into monosaccharides are added to the system (Xu et al. 2020). Studies on ensiling have been conducted over different ensiling periods such as 90 days (Zheng et al. 2014), 180 days (Hillion et al. 2018; Gallegos et al. 2018), and even as short as 28 days (Zheng et al. 2012a). All these studies showed that ensiling is a good method to preserve wet biomass for biofuel production. However, for seasonal substrates like SBL, there is a need for longer period of ensiling if it is to be used as a substrate for sustainable biogas production.

Most of the studies on ensiling reported in literature were carried out on a laboratory scale. However, there are some challenges in transferring the results from the laboratory to practical application. Laboratory experiments are conducted in small containments (e.g. airtight bags, silage jars, etc.), which allows for replication and controlled conditions. However, such laboratory experiments do not take into account the influence of gravity on silages. In practice, gravity may influence the distribution of TS, VS and fermentation parameters within the different areas (Heidarzadeh Vazifehkhoran et al. 2016). This might be a reason for differences between laboratory scale and practice. Another aspect may be the type of harvesting. For laboratory experiments and the small quantities needed for them, it is much easier to use hand-harvested leaves than to coordinate sampling and laboratory work with a machine-harvesting schedule. However, hand harvesting typically yields only leaves (without beet tops) and involves less mechanical treatment of the leaves. The composition of machine and hand-harvested materials may therefore differ in TS, VS, soluble carbohydrates (due to the higher sugar content in the beet tops), nitrogen (N) content and buffer capacity (Kilmartin and Oberholster 2022). Microbial degradation may start faster in the machine-harvested material due to the shredding effect at harvest, thereby leading to fermentation even before ensiling. In addition, silages are usually exposed to air during feed-out to reactors, a situation that can reduce the quality of ensiling (Kung et al. 2018). Ensiling substrates in silos that can enable the transfer of silages from the silos to reactors without being exposed to air will ensure that the stability of the silages is not compromised and is therefore the focus of this research.

For a sustainable biomethane production from SBL, it is necessary to understand the overall process performance of the bioreactor for an efficient control of process parameters needed for an optimal biomethane yield (López-Pérez et al. 2018). One of the ways of describing the overall performance of a bioreactor is by the use of kinetic models (López-Pérez et al. 2016). Parameters obtained from kinetic models that describe a given biochemical process are sometimes specific to substrate types and process parameters (Romero Cortes et al. 2018). To the best of our knowledge, there is no available literature on the kinetic study of biomethane potential from SBL.

The aim of this study was to (i) investigate the quality of ensiled sugar beet leaves stored in vertical columns and in airtight bags at ambient temperature, thereby enabling the study of changes in the properties of the silages due to gravity as a function of the height of the columns (ii) determine the biomethane potential of the different silages (iii) estimate the theoretical biomethane potential of the silages using their elemental composition (iv) describe the kinetics of the biomethane potential by fitting the experimental biomethane potential to four selected models: the first-order kinetic model, the Monod-type kinetic model, the one-step-two-fractions model and the modified Gompertz model. To determine if the harvesting method had an influence on the quality of ensiling and methane potential of the substrates, some of the leaves were harvested by hand, while the others were harvested with a machine and then ensiled in sealed plastic bags. An additive was also added to some of the silages in the airtight bags to test its effect on ensiling quality and BMP.

Materials and Methods

Sugar beet leaves were harvested in mid-October 2018 at a local farm in Saxony, Germany. Some of the SBL were harvested by hand, while the rest was harvested by using a machine (ROPA euro-Tiger Beet harvester, Germany). Both the hand-harvested leaves (HH) and the machine-harvested leaves (MH) were transported separately in large plastic bags to the laboratory and left inside the bags at ambient temperature for 24 hrs before processing. The additive used for ensiling was a commercially available homofermentative lactic acid bacteria produced in September 2014 and supplied by *Schaumann Bioenergy GmbH,* Germany. The additive was mixed with the substrate according to the manufacturer’s specification.

The inoculum (pH = 7.65, TS = 2.64 %, VS = 71.87 %TS) used for the BMP tests was obtained from an active continuous stirred tank reactor (CSTR) located at the Deutsches Biomasseforschungszentrum gGmbH (DBFZ), Germany. The CSTR is specifically operated to provide inoculum for BMP testing and runs on a maize silage-cattle manure-sunflower oil mixture with a hydraulic retention time of 100 days and an organic loading rate of 0.5 gVS/L/d.

Ensiling

Figure 1 shows the workflow from harvest to BMP tests. The treatment of the leaves started 24 hrs after harvest. Both the HH and MH were separately shredded using a mechanical grinder (Mainca PC-82/22, Spain) to an average particle size of 1.4 mm. The shredded MH were divided into three portions. One portion was stored in triplicate vertical columns each with a height of 3.60 m and internal diameter of 0.1536 m. The vertical columns were made of polyvinyl chloride with outlet taps that could be opened and closed at five different points for sampling (Figure 2). The columns were then sealed at the top with a 0.40 m diameter lid. The headspaces were flushed with nitrogen for about 1 minute and the batches were stored at ambient temperature for 370 days. A second portion of MH was mixed with the additive and referred to as MHA. The third portion of the MH as well as the HH were ensiled without additive. About 300 g of shredded HH, MH (third portion) and MHA were separately packed in triplicates into 310 × 105 mm airtight bags (PA/PE, La.va, Germany), vacuum sealed to remove oxygen, and also stored at ambient temperature for 370 days.

Sampling and sample preparation

For analyses of the silages in the bags, triplicate batches of each of HH, MH, and MHA were sacrificed. The contents of the replicate bags were placed in a 3-L bowl and mixed thoroughly with a spoon for about 1 minute to generate a homogenous sample. For the analysis of silages in the vertical columns, samples were taken from five outlet points (heights of 3.40, 2.60, 1.82, 1.0 and 0.20 m; see Figure 2). About 300 g of sample from each sampling point of the columns was put into a 3-L bowl and mixed thoroughly. Samples were taken from the bowl in triplicate for analysis.

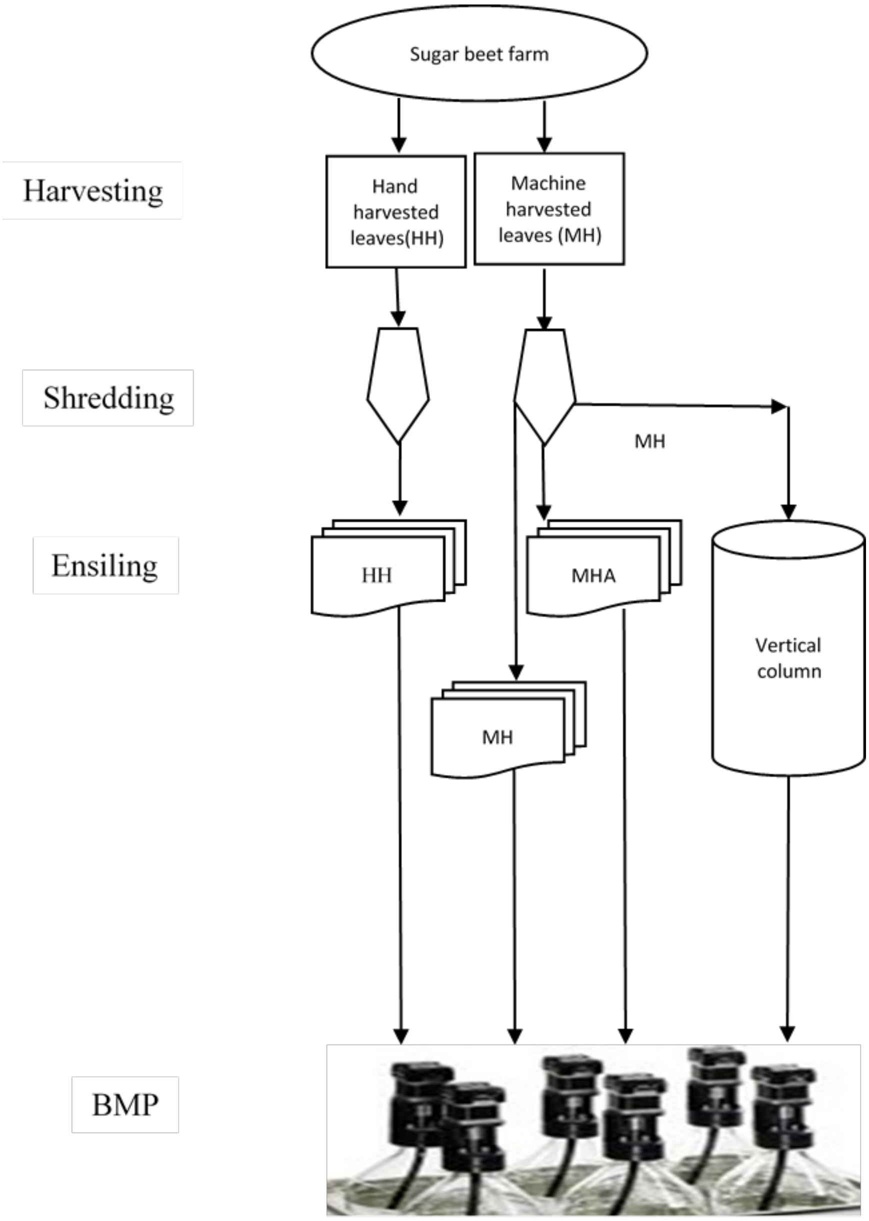

Fig. 1. Experimental approach: HH, hand-harvested leaves; MH, machine-harvested leaves; MHA, machine-harvested leaves with additives; BMP, biomethane potential.

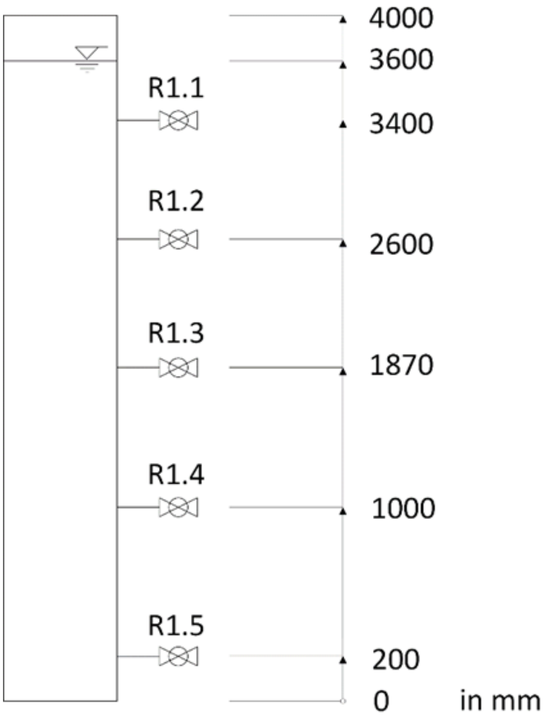

Fig. 2. Schematic diagram of the vertical columns. R1.1, R1.2, R1.3, R1.4 and R1.5 represent the exit points 3.40 m, 2.60 m, 1.87 m, 1.00 m and 0.20 m respectively.

Analytics

The TS and VS contents of the fresh leaves and silages were determined using standard methods (Strach 2016). To account for the loss of volatile acids and alcohols during analyses, the values of TS and VS were corrected using appropriate equations (Weissbach and Strubelt 2008). Prior to the determination of the pH and the analyses of fermentation quality of the fresh leaves and silages, each sample was mixed with deionized water in a ratio of 1:10 (w/w) and shaken (Innova 44, New Brunswick Scientific) at 110 rpm and 5 °C for 24 hrs. The mixtures were centrifuged (Heraeus Megafuge 16R, Thermo Fischer Scientific, Waltham, USA) at 10,000 × g for 10 minutes and the supernatant was filtered with a 0.1 µm filter (MilliporeSigma, Canada). The filtrate was used to measure the pH using a Sen Tix 41 pH electrode (WTW, Germany) and to determine the concentrations of ammonia nitrogen (NH_3-N), water-soluble carbohydrates (WSC), organic acids and alcohols. WSC were analysed using an Azura HPLC system (Knauer GmbH, Germany) equipped with degasser, binary pump system, auto sampler, column oven, and refractive index detector (RID) set at 40 °C following the method of Mühlenberg (2016). The contents of volatile fatty acids (VFA) and alcohols were measured by analysis on a 7890A gas chromatograph with a flame ionization detector (FID) (Agilent Technologies, USA) as described by Apelt (2016). NH_3-N concentration was determined using tests kits, Nessler reagent and Hack DR 2000 spectrophotometer (HACK LANGE GmbH , Germany). Carbon (C), hydrogen (H) and nitrogen (N) content of the silages were determined by means of an elemental analyser (TrueSpec, LECO Instrumente GmbH, Mönchengladbrach, Germany). Oxygen (O) content was calculated based on the ash content and analysed elemental composition.

Biomethane potential assay

The BMPs of all silages were measured using the AMPTS II (Bioprocess control, Sweden). Details of the procedure were described previously (Sträuber et al. 2015). Inoculum to substrate ratio (ISR) was 3.2 (VS basis) for all silages. Additionally, a triplicate positive control consisting of 2.85 gTS of microcrystalline cellulose (MCC) as well as triplicate negative control consisting of 400 mL of only inoculum were used to monitor the quality of the inoculum. The headspace of each reactor was flushed with nitrogen for about 2 min to ensure anaerobic conditions. Daily methane production of each reactor was recorded using the software Bioprocess Control (Lund, Sweden). The batch tests were conducted at a mesophilic temperature of 38 °C for 31 days with the silages from the bags, and 25 days with the silages from the vertical columns. In each case, experiment was terminated when the daily BMP over three consecutive days was less than 1 % of the accumulated BMP in accordance with VDI 4630 guideline.

Kinetics of biomethane production and data analysis

Four kinetic models were used to fit the cumulative methane production data. These models were the pseudo-first order model (FOKM) as given in Equation 1, the Monod-type kinetic model given in Equation 2, the one-step-two-fraction model (OSTF) given in Equation 3, and the modified Gompertz model given in Equation 4. These equations were chosen because (i) the first-order model and the Monod-type kinetic models were necessary to obtain hydrolytic rate constants, k (ii) the one-step-two fraction model would properly account for the fast and slow degradable components of SBL and (iii) the modified Gompertz model would provide insight into the lag phase and maximum rate of biomethane production of the silages. Although the Monod equation has its limitaions, it was used in the present study because it is the most widely used model in describing substrate utilization rate (Muloiwa et al. 2020). Parameters from the models were determined using a Solver add-in program in Microsoft Excel software (version 2016, Microsft Corporation). SigmaPlot software (version 7.0) was employed to design kinetic figures. The correlation coefficient (R^2), root mean square error (RMSE) given in Equation 5, the Bayesian Information Criterion (BIC) given in Equation 6, and the Akaike Information Criterion (AIC) given in Equation 7 were the statistical indicators used to determine the fitness of the models to the experimental data. All data were analysed with Microsoft Excel using the Two-Way Analysis of Variance (ANOVA). Tukey's post hoc test was adopted to compare differences at 0.05 significant level. Pearson correlation analysis was used to evaluate the interdependence of fermentation products.

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

$$\mathrm{G_{(t)}} = \mathrm{G_{(0)}}\left(\frac{\mathrm{kt}}{1+\mathrm{kt}}\right) \tag{2}$$

$$\mathrm{G_{(t)}} = \mathrm{G_{(0)}}[1 - \alpha \mathrm{e}^{-\mathrm{k_1 t}} - (1 - \propto)\mathrm{e}^{-\mathrm{k_2 t}}] \tag{3}$$

$$G_{(t)} = G_{(0)}.\exp\{-\exp\left[\frac{R_{max}.e}{G_{(0)}}(\lambda - t) + 1\right]\} \tag{4}$$

where $G_{(t)}$ is the cumulative methane potential (mL/gVS) at time point t, G_0 is the maximum possible methane potential at a theoretically infinite digestion time (mL/gVS), R_{max} is the maximum methane production rate (mL/gVS/d), λ is the lag phase time (d), *e* is the Euler's number (2.7183), k_1 is the first-order degradation constant of fast degradable substrate (1/d), k_2 is the first-order degradation constant of slow degradable substrate (1/d), α is the fraction of readily degradable material, *t* is the duration of digestion (d), and *k* is the first-order reaction rate constant (1/d).

$$RMSE = \sqrt{\frac{ss}{n}} \tag{5}$$

$$BIC = nln\left(\frac{ss}{n}\right) + kln(n) \tag{6}$$

$$AIC = n \times \ln\left(\frac{ss}{n}\right) + 2k + \frac{2k(k+1)}{n-k-1} \tag{7}$$

where *n* is the number of experimental data, *ss* is the squared sum of residuals, and *k* is the number of parameters in the model.

Theoretical biomethane potential and biodegradability

Using the elemental composition, the theoretical biomethane potential (TBMP) of the silages was determined at standard conditions using the Buswell’s formula given in equations 8 and 9.

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \tag{8}$$

$$TMP\left(\frac{mL}{gVS}\right) = 22415\,\frac{\frac{4a+b-2c-3d}{8}}{12a+b+16c+14d} \tag{9}$$

The biodegradability (BD) of the silages was calculated using equation 10.

$$BD\ (\%) = \frac{Experimental\ methane\ potential}{Theoretical\ methane\ potential} \tag{10}$$

Results and Discussion

Characteristics of the feedstock

The characteristics of the SBL before ensiling are presented in Table 1. TS and VS contents of HH were consistent with the report of Kreuger et al. (2011) and Larsen et al. (2017). The VS content of the MH and MHA substrates were significantly higher ($p<0.05$) than those of HH, probably because of the presence of sugar beet tops and other grasses that accompanied the MH during harvest. Glucose was the only component of WSC present in the leaves, as xylose and fructose were absent. The high concentration of WSC in the substrate is an indication that SBL is a good substrate for ensiling. The presence of lactic acid, acetic acid and ethanol in the substrates is an indication of initial fermentation before ensiling. Higher values of fermentation products in the MH could mean that fermentation started earlier in the substrate than in the HH.

Table 1. Physico-chemical parameters of substrates before ensiling (±standard deviation)

Parameter	Substrates		
	HH	**MH**	**MHA**
TS (%)	13.49 ± 0.07	14.57 ± 0.06	14.64 ± 0.07
VS (%TS)	83.17 ± 0.12	84.35 ± 0.09	84.40 ± 0.08
WSC (g/L)	72.27 ± 5.31	76.02 ± 4.7	n.d
Acetic acid (g/L)	3.21 ± 0.02	7.56 ± 0.01	n.d
Lactic acid (g/L)	6.52 ± 0.00	9.76 ± 0.01	n.d
Butyric acid(g/L)	< 0.01	< 0.01	n.d
Ethanol (g/l)	0.85 ± 0.00	2.23 ± 0.00	n.d
pH	4.94 ± 0.00	4.89 ± 0.04	n.d

n.d, not determined

Quality of Ensiling

TS, VS, pH, and concentration of fermentation products in the silages from the columns after 370 days of ensiling are shown in Table 2. Silages from point R1.5 had significantly higher ($p<0.05$) TS and VS values than silages from point R1.1, probably because the materials tend to move towards gravity. This trend of a progressive increase in TS and VS of silages with increasing depth of a silo has also been reported by Heidarzadeh Vazifehkhoran et al. (2016) in the ensiling of sugar beets. Higher TS and VS in the lower parts of the columns is a result of compression due to the effect of gravity. Compression reduces pore volumes in the columns and makes for a better ensiling. For the silages in the bags, the VS loss in HH silage (11.81 %) was significantly lower ($p<0.05$) than the VS losses in the MH (16.30 %) and MHA (16.39 %) silages respectively, indicating that the method of harvesting sugar beet leaves significantly affects VS losses during ensiling (Table 3). VS losses during ensiling is a consequence of the formation of fermentation products and has also been reported from the ensiling of whole rye (Auerbach et al. 2020) and maize stover (Sun et al. 2020). The higher VS losses in MH and MHA silages compared to HH silage may be due to the production of more fermentation products arising from the presence of other components like sugar beet tops. Comparable losses in VS (16.74 %) has been reported by Larsen et al. (2017) after nine months of ensiling sugar beet tops mostly at 20 °C.

Table 2. Effect of different heights of the columns on quality of ensiled sugar beet leaves (±standard deviation)

Parameter	R1.1	R1.2	R1.3	R1.4	R1.5
TS	12.90±0.92	13.10±1.32	14.51±1.47	14.70±1.77	14.90±1.57
VS	72.41±1.34	73.73±1.83	73.55±0.89	74.64±2.24	75.13±1.32
LA	4.23±0.37	12.28±0.29	12.07±0.17	12.02±1.32	12.17±1.28
AA	2.42±0.11	3.84±0.28	3.83±0.03	3.70±0.07	3.55±0.92
PA	1.04±0.07	2.01±0.02	2.39±0.14	2.45±0.11	2.51±0.03
Eth	1.15±0.02	2.66±0.04	2.78±0.03	2.81±0.00	2.83±0.06
Pro-ol	0.00	0.38±0.01	0.57±0.13	0.68±0.04	0.68±0.24
TFP	13.86±1.33	21.17±1.19	21.64±1.91	21.66±1.48	21.74±1.37
% LA	47.85±2.17	58.01±2.12	55.78±0.97	55.49±1.97	55.98±1.22
pH	4.68±0.26	4.23±0.17	4.28±0.19	4.27±0.21	4.28±0.33
C	38.15±0.07	n.d	38.13±0.08	n.d	38.20±0.87
H	4.70±0.02	n.d	4.69±0.05	n.d	4.71±0.08
N	3.95±0.02	n.d	3.92±0.07	n.d	3.92±0.01
O	26.23 ±0.09	n.d	26.81±1.01	n.d	28.30±1.19

Table 3: Fermentation products of silages from bags

					p-value		
Parameter	HH	MH	MHA	SEM	H	A	H×A
TS(%FM)	12.15	12.74	13.13	0,13	<0.001	<0.001	<0.001
VS(%TS)	73.35	70.60	70.65	0.14	0.035	0.134	0.071
LA(g/L)	17.53	17.22	17.19	0.42	0.812	0.867	0.9688
AA(g/L)	3.21	6.11	9.35	0.27	<0.01	<0.01	0.01
BA(g/L)	0.01	0.03	0.75	0.017	0.673	<0.001	0.748
PAA(g/L)	0.22	0.28	0.55	0.05	0.358	<0.001	0.437
Eth(g/L)	1.15	2.56	3.78	0.09	<0.001	<0.01	<0.001
%LA	79.29	65.55	54.36	0.91	0.0669	0.072	0.058
TFP(g/L)	22.11	26.27	31.62	0.51	0.563	0.3457	0.526
C(%TS)	36.87	38.17	38.18	0.036	<0.001	0.3905	0.672
H(%TS)	4.61	4.65	4.66	0.005	0.679	0.218	0.769
N(%TS)	3.00	3.97	3.98	0.005	0.027	0.165	0.218
O(%TS)	28.87	23.81	23.83	0.021	<0.01	0.281	0.287

FM, fresh matter; TS, total solids; VS, volatile solids; LA, lactic acid; AA, acetic acid; BA, benzoic acid; PPA, phenylacetic acid; Eth, ethanol; TFP, total fermentation products; C, carbon; H, hydrogen; N, nitrogen; O, oxygen; SEM, standard error of mean; H, effect of method of harvesting; A, effect of additive; H×A, interaction effect between method of harvesting and additive.

An important parameter that is used as an indicator of silage quality is the pH (Kafle et al. 2013). In our study, the pH was in the range of 4.06 – 4.68 for the silages from the columns, with the highest pH being from silages from point R1.1 and the lowest being from silages from point R1.5. This difference in pH with respect to the position of the silages in the columns could be attributed to differences in pressure-heights in the columns. The increase in pressure with depth of column leads to an increase in the density of the silages at the various points, resulting in better ensiling. He et al. (2021) has also reported an inverse relationship between density and pH in the ensiling of *Broussonetia papyrifera* leaves. For the silages in the bags, the pH was 3.91 for the HH silage, 3.95 for the MH silage, and 4.12 for the MHA silage. Depending on the type of silage and TS content, the pH of a good silage should be between 3.7 and 5.0 (Rooke and Hatfield 2003). Considering the TS values of all silages in our study, the pH ranges were evidence of good ensiling and were consistent with the report of Kafle and Kim (2013). A higher pH of 5.18 was reported by Hillion et al. (2018) after nine months of ensiling SBL at 15 °C. However, Zheng et al. (2012b) reported a pH between 3.91 and 4.03 after three months of ensiling sugar beet leaves at 22 °C. Differences in pH of silages can be influenced by factors like handling technique (e.g duration between harvesting and ensiling) of substrates before ensiling.

The low pH values obtained in our study as well as the values reported in literature are the result of lactic acid production in the systems (Yang et al. 2019). As shown in Tables 2 and 3, the predominant fermentation product in all silages was lactic acid as has been reported for many other good silages (Franco et al. 2018). Except for the silages from point R1.1, the lactic acid content in all silages was above 55 % of the total fermentation products, which is consistent with the recommended amount for grass silage (Kung et al. 2018). The significantly lower ($p<0.05$) concentration of lactic acid in silages from point R1.1 compared to silages from other points of the column could be attributed to the movement of materials down the height of the column as a result of the influence of gravity. Beyond point R1.1, the height of the columns had no significant effect ($p<0.05$) on the lactic acid concentration. Similarly, neither the treatment, harvesting method nor the interaction between the treatment and harvesting method had a significant effect on the lactic acid concentration in the HH, MH and MHA silages (Table 3) despite the higher concentration of WSC initialy present in MH (Table 1).

Acetic acid concentration was significantly lower ($p<0.01$) in silages from R1.1 compared to other points of the columns, an indication that silages at the top will have lower stability during feed-out. Beyond point R1.1, acetic acid was not significantly different at any point in the columns. Conversely, the treatment, harvesting method and the interaction between treatment and harvesting method had a significant effect ($p<0.01$) on the acetic acid concentration in the silages from the bags (Table 3), indicating that the substrates that would be more stable when exposed to air during feed-out is in the order of MHA>MH>HH. The higher concentration of acetic acid in the MH silage compared to the HH silage could be attributed to the higher concentration of WSC initially present in the MH.

In addition to lactic and acetic acid, propionic acid was also present in the silages from the columns, with its concentration increasing down the column. Propionic acid was not detected in the silages from the bags. Instead, traces of benzoic acid and phenylacetic acid were found in these silages, but not observed in the silages from the columns, indicating that the conditions of ensiling influenced the type of acids formed during ensiling, as also reported by Kalač (2017). The concentrations of benzoic acid and phenylacetic acid were influenced by the treatment but not by harvesting method, consistent with the report of Liu et al. (2018) who reported a significant difference in the concentration of benzoic acid in oat silage with and without additive, indicating that the additive used in our study favoured benzoic acid and phenylacetic acid production.

Ethanol concentration was also significantly lower in silages from point R1.1 compared to silages from other points of the columns, probably because of a depletion in materials at that point due to gravity effect. Beyond point R1.1, ethanol concentration was not significantly different in the silages, an indication that yeast activity, which enhances ethanol production during ensiling (Kung et al. 2018), was not affected by the height of the columns. However, ethanol concentrations in the silages from the bags were statistically different, indicating that the treatment, harvesting method (arising from the presence of sugar beet tops) and the interaction between treatment and harvesting method affected the yeast activity during ensiling. The ethanol concentration in the bags was highest in the MHA silage and lowest in the HH silage.

Except for the silages from point R1.1, all other silages from the columns contained Propan-1-ol. The formation of Propan-1-ol during ensiling has been associated with higher VS losses (Wang et al. 2014), which results from the degradation of lactic acid. Propan-1-ol was not present in the silages from the bags probably because the condition (presence of void volumes and absence of compaction) did not favour its production. As expected, the concentrations of total fermentation products (TFP) were significantly higher ($p<0.05$) at points R1.5 compared to points R1.1 in the columns. For the silages from the bags, TFP was not significantly different, indicating that neither the treatment nor the harvesting method had a significant effect on the TFP concentration.

Although SBL contains 22.8 % (VS basis) crude protein (Tenorio 2017), the NH_3-N content in all silages was less than 0.01 mg/L (not shown), indicating that no protein degradation occurred (Herremans et al. 2019), another indication of adequate ensiling. In contrast to our observations, the NH_3-N content of maize silage was shown to increase from 6.2 % of total nitrogen after 49 days of ensiling to 7.4 % of total nitrogen after 90 days of ensiling without additives at a pH of 3.80 (Herrmann et al. 2015). Some of the additives used by the authors (Herrmann et al. 2015) increased the NH_3-N content of the silages by up to 68 % within 41 days at a pH range of 4.20-4.30, suggesting that the type of additive may affect protein degradation. In our study, neither column height, harvesting method, nor treatment had any effect on the NH_3-N content.

There was no significant difference in the elemental composition of the silages from the column. Although there was a movement of material from the top to the lower parts of the columns, this gravity effect did not affect the elemental composition of the silages, probably because elemental composition is an intensive property of a material and therefore depends only on the nature of a substrate and not on the amount of available substrates. For the silages from the bags, the carbon and nitrogen content of the HH silage was significantly lower compared to the values from the MH and MHA silages, probably due to the presence of materials other than SBL like sugar beet tops and grasses that accompanied the MH. Conversely, treatment had no effect on the elemental composition of the silages, indicating that the additive only aided the formation of fermentation products from WSC but did not affect the elemental composition of the silages.

Overall, as far as the stability of the silage is concerned, gravity had a positive effect on the silages beyond point R1.1 based on the higher concentration of propionic acid and acetic acid. Since the VS in the columns were significantly different from one point to another, changes in process parameters like organic loading rate may be required when the silage is to be used for anaerobic digestion (AD). The silage quality at point R1.1 may be improved by reducing the head space volume in the columns (Franco et al. 2017). This can be done by filling the top of the columns with an inert material. Based on the pH, percentage composition of lactic acid and absence of butyric acid, ensiling can be said to be adequate for all silages.

Biomethane Potential Results

In addition to the silages from the bags, only silages from points R1.1, R1.2 and R1.5 were used for the BMP tests. These points corresponded to heights 3.40, 1.87 and 0.20 m respectively and were considered representative of the entire column. The highest cumulative BMP for the silages in the columns was measured with the sample from point R1.5. The BMP measurements of all silages showed the same pattern (Figure 3), with more than 74% of the methane produced in the first three days of AD. In a boxplot (not shown), the methane produced on the first three days appeared as outliers, indicating that the biomethane production rate on these days was much higher than the rate on subsequent days. The silage from point R1.1 had the lowest BMP, probably because more degradable substrates had gravitated down the column. The daily production of biomethane declined for all substrates until the end of the experiments. This can be attributed to the depletion in the amount of degradable compounds (Nguyen et al. 2019). There was a significant difference in the BMPs of silage from point R1.1 and the lower parts of the column (R1.3 and R1.5), which is consistent with the report on sugar beet pulp silo (Heidarzadeh Vazifehkhoran et al. 2016) and is important for the practical management of such silage stores. In addition, Lower concentration of precursors to biomethane production like acetic acid as well as lower concentration of products with high theoretical BMP (ethanol, propan-1-ol and propionic acid) in the silage from point R1.1 may have also contributed to its lower BMP. The BMPs of silages from points R1.3 (344.81±7 mL/gVS) and R1.5 (364.49±20 mL/gVS) were not significantly different, but were significantly higher ($p<0.05$) than the BMP of silage from point R1.1 (300.66±3 mL/gVS).

The cumulative BMPs of HH (337.24±3 mL/gVS), MH(339.04±10 mL/gVS), and MHA(364.60±8 mL/gVS) silages were not significantly different ($p<0.05$), indicating that neither the treatment nor harvesting method had a significant effect on the BMP of the silages, as has been reported for maize silage (Vitez et al. 2021), indicating that the sugar beet tops and other grasses as part of the MH did not enhance the methane production. In fact, had the sugar beet tops been present in a much larger quantity, the BMP might have been lower (Wróbel et al. 2020). A comparison of the BMPs of SBL in our study with those of other studies is summarised in Table 4. Differences between sugar beet species, ensiling conditions, and additives used may affect BMP (Kung et al. 2018) of SBL. Although not statistically significant, the additive used increased the BMP of MHA silage by about 8% compared to MH silage. If this additive is to be used in the ensiling process, a trade-off must be made between the cost of the additive and the gain resulting from the 8% increase in biomethane production.

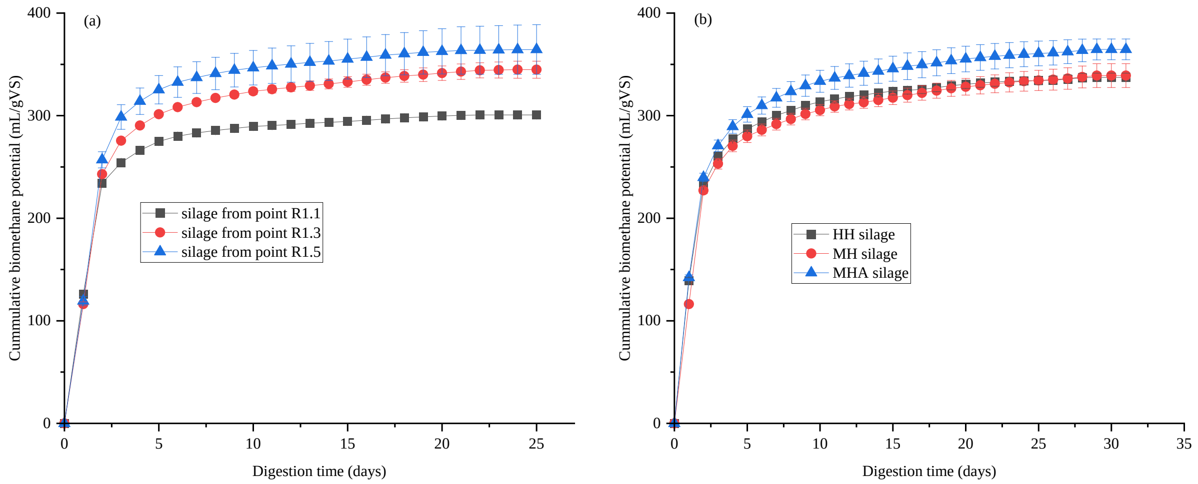

Figure 3. Cummulative biomethane potential of silages from (a) column and (b) bags

Table 4. Comparison of biomethane potential values of sugar beet leaves

Nature of Substrates	BMP (mL/gVS)	*Scale of operation	Reference
Fresh	210	Lab	(Amon et al. 2007)
Fresh	361	Lab	(Gissén et al. 2014)
Ensiled (61 days)	440	Lab	(Larsen et al. 2017)
Fresh	313	Pilot	(Larsen et al. 2017)
Ensiled (column) (370 days)	364	Pilot	This study
Ensiled (bags) (370 days)	365	Lab	This study

BMP, biomethane potential; Lab, laboratory. *Scale of operation relates to the ensiling process and not the biomethane potential determination.

Theoretical biomethane potential

The theoretical biomethane potential (TBMP) and biodegradability (BD) of the silages is shown in Figure 4. All silages had a higher TBMP compared to the measured BMP, probably because both the biodegradable and non-degradable components of the substrates were assumed to be converted to methane during the calculation of TBMP. For the silages in the column, the BD increased towards the depth of the column, a confirmation that digestable fraction of the substrates moved under gravity towards the bottom of the column. For the silages in the bags, HH silage had the highest BD, possibly because the non-SBL components that accompanied the MH silage had higher non-degradable fractions than SBL.The BD of MHA was slightly higher than that of MH, indicating that the additive had a positive influence on the BD of sugar beet leaves. Overall, the BD of SBL compares with those of cattle manure and organic wastes from cattle rumen (Nguyen et al. 2019).

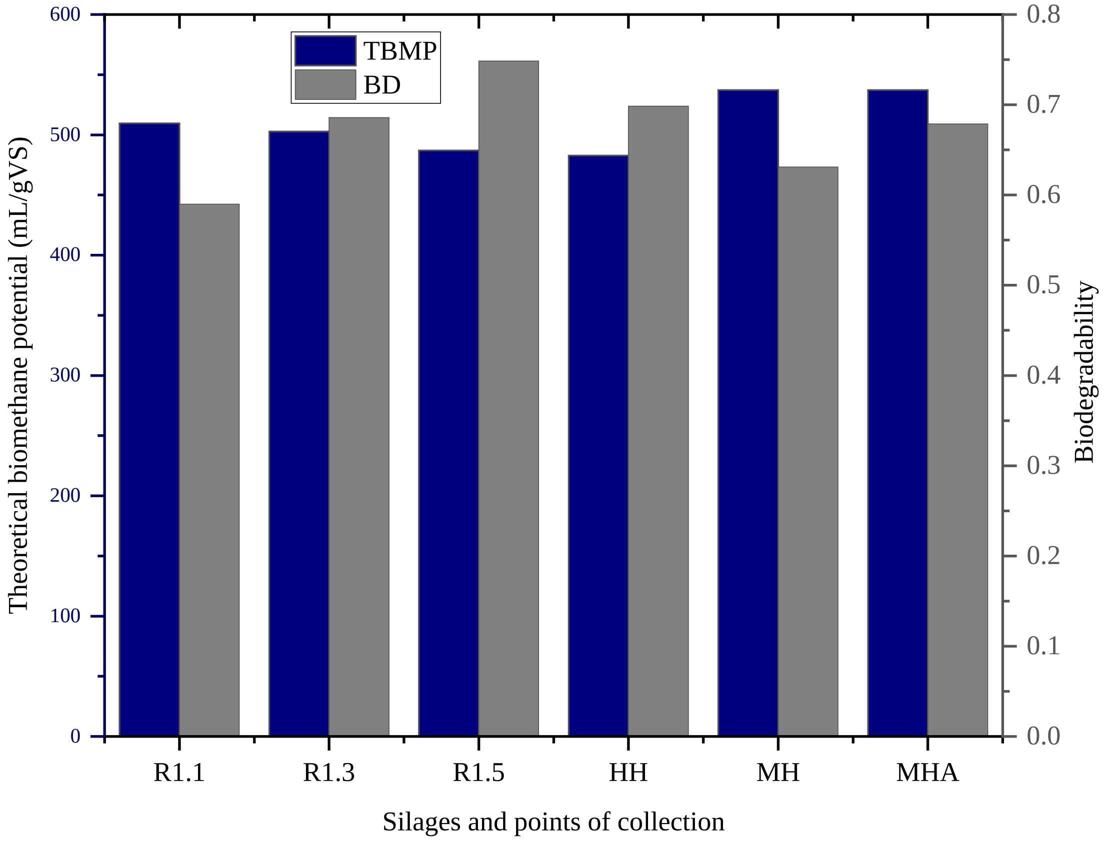

Figure 4. Theoretical biomethane potential and biodegradability of silages

Kinetic Models and Validation

All kinetic models provided a good degree of fitness to the experimental data, as shown by the high R^2 values (Table 5). For all silages, the predicted methane yield from the modified Gompertz model was lower than the predicted methane yield from the other models. The best model can be defined as the one with the lowest values for AIC, BIC, and RMSE (Nguyen et al. 2019) and the highest R^2 values. From Table 5, it can be seen that the one-step-two-fraction kinetic model (OSTF) fitted the experimental BMP of all silages better than the other models, probably because the model was developed on the assumption that substrates for AD can contain both fast and slow degradable components (Brulé et al. 2014). The fitness of the OSTF model to the experimental data is shown in Figure 5. The fraction of readily degradable materials, α, was significantly lower in R1.1 compared to other points (R1.3 and R1.5) of the column probably due to gravity effect. Silages from the bags had higher α values than silages from the column, which may have resulted in the longer duration of AD (31 and 25 days for the silages from the bags and column respectively) of the substrates. The modified Gompertz model had the highest RMSE and the lowest coefficient of determination (0.94 – 0.98) for all samples, probably because of the absence of a lag phase. Strömberg et al. (2015) has also reported the poor performance of the Gompertz model in describing the AD of household waste, sewage sludge, lipid-rich waste, and even some agricultural residues such as bamboo waste and banana peels. However, the equation can provide information on the lag phase, λ of an AD process. In our study, the λ was 0 h for the silages from the bags and an average of less than 2 h for the silages from the column. This slight difference in λ could be due to the difference in the nature of fermentation products in the silages.

The maximum methane production rate, R_{max}, was higher in silages from the column, probably because these substrates had higher values of k (Mao et al. 2017), which is an indication of faster degradability. 90 % of the cumulative methane of the silages from the column was produced in the first five days of AD. This time, beyond the lag phase, during which 90 % of the gas was produced, is called the effective biomethane production time, T_{eff}

(Mao et al. 2017). T_{eff} for HH, MH, and MHA was approximately 9 days, while T_{eff} for the column silages was approximately 5 days, which correlates with α values. The T_{eff} value of the OSTF model closely matched the T_{eff} value of the measured data, also indicating that the model predicted the BMP better than the other models. In Pramanik et al. (2019), a T_{eff} value of 53 days was reported for food waste using the modified Gompertz model. In our study, the modified Gompertz model gave a T_{eff} of 4 days for all silages except for the silage from point R1.1, where the T_{eff} was 3 days probably due to lower α. The kinetic constants of the rapidly degradable fractions (k_1) of the silages determined from the OSTF were at least 8 times higher than the slowly degradable fractions (k_2), which is consistent with the report of Gallegos et al. (2018). The range of hydrolytic constants (k) from the Monod-type kinetic model (0.1129 – 0.1154 /d) was lower than the range (0.2619 – 0.2639 /day) obtained from the first order model, indicating that kinetic constants can vary even for the same substrates depending on the kinetic model used. However, the hydrolytic constants obtained in our study are consistent with the range of 0.128-0.238 /d reported for sugar beet tops after 210 days of ensiling (Ohuchi et al. 2015). In both the first-order and Monod-type kinetic models, k values of silages from the columns at all points were similar and were not significantly different from k values of silages from the bags despite the difference in concentration of organic acids, probably because all reactors were operated at the same temperature. Fogler (2016) has reported that k values are significantly affected by temperature and that concentration of fermentation products has little effect on it.

Table 5. Estimated Parameters of the models and their statistical significance

Parameter	**Silage/collection point**					
	HH	**MH**	**MHA**	**R1.1**	**R1.3**	**R1.5**
First-order kinetic model (FOKM)						

R^2	0.9672	0.9786	0.9734	0.9854	0.9825	0.9884
AIC	161.53	146.74	159.01	102.35	121.04	119.72
BIC	163.97	149.18	161.45	104.24	122.93	121.62
RMSE	12.60	9.93	12.10	9.56	10.28	10.01
G_0 (mL/gVS)	326.29	323.99	350.64	294.57	334.30	356.43
$k(d^{-1})$	0.2639	0.2619	0.2632	0.2621	0.2617	0.2628
Monod type model						
R^2	0.9880	0.9947	0.9958	0.9788	0.9789	0.9728
AIC	130.44	103.40	101.72	117.42	125.69	135.33
BIC	132.88	105.84	104.16	119.31	127.58	137.22
RMSE	7.63	4.94	4.80	9.56	11.28	13.68
G_0 (mL/gVS)	337.70	336.50	363.65	304.41	347.17	369.85
$k(d^{-1})$	0.1145	0.1131	0.1154	0.1142	0.1138	0.1129
One-step-two-fractions model (OSTF)						
R^2	0.99	1.00	1.00	0.99	0.99	0.99
AIC	107.68	67.97	70.53	89.24	104.29	111.11
BIC	115.88	76.17	78.73	91.08	106.13	112.94
RMSE	5.19	2.74	2.85	5.44	7.34	8.42
G_0 (mL/gVS)	342.30	361.96	371.91	356.68	478.71	498.46
$k_1(d^{-1})$	0.754	0.731	0.747	0.728	0.719	0.723

$k_2 (d^{-1})$	0.087	0.090	0.085	0.067	0.059	0.049
α	0.89	0.77	0.81	0.64	0.80	0.85
Modified Gompertz model						
R^2	0.9428	0.9529	0.9427	0.9807	0.9722	0.9813
AIC	179.26	171.62	183.25	114.24	132.57	126.78
BIC	184.67	177.03	188.67	116.11	134.43	128.64
RMSE	16.65	14.72	17.76	8.97	12.94	11.52
G_0 (mL/gVS)	323.15	320.22	346.76	292.05	330.50	352.49
R_{max} (mL/gVS/d)	75.84	83.43	85.98	103.56	119.17	133.10
λ (d)	0.00	0.00	0.00	0.11	0.04	0.08

HH; hand-harvested leaves, MH; machine-harvested leaves; MHA; machine-harvested leaves with additive; G_0, maximum methane yield at a theoretically infinite digestion time; R_{max}, maximum methane production rate; λ, lag phase time; k_1, first-order degradation constant of fast degradable substrate; k_2, first-order degradation constant of slow degradable substrate; α, fraction of readily degradable material.

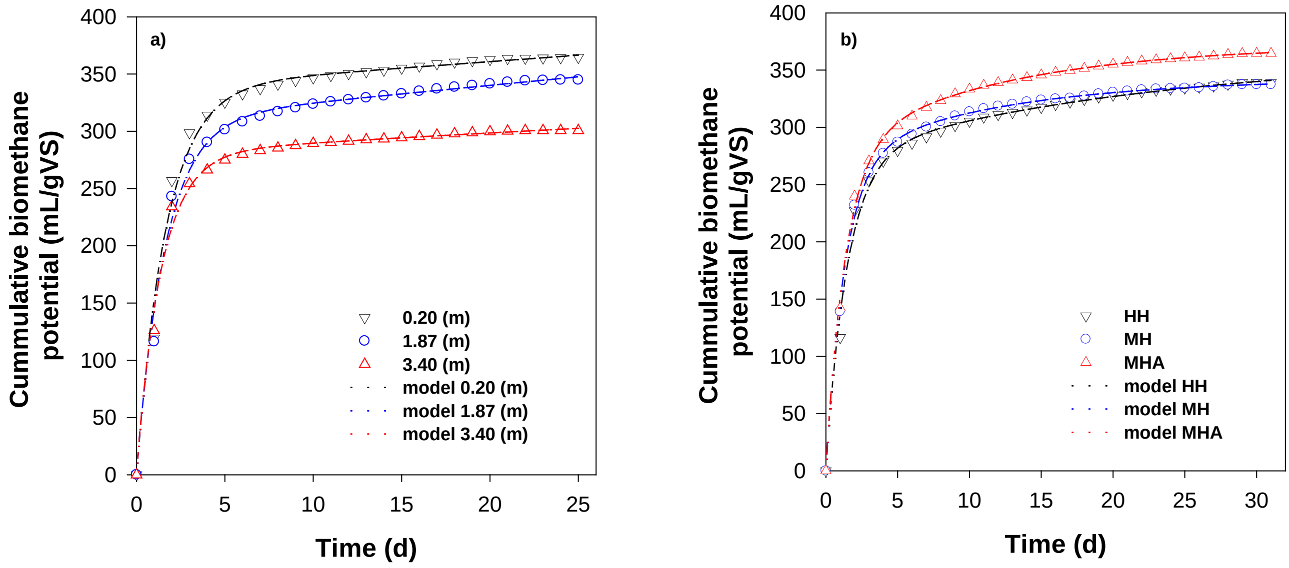

Figure 5. Kinetic modelling of the biomethane potential of silages from (a) column and (b) bags

Conclusions

Sugar beet leaves (SBL) have proven to be a suitable substrate for ensiling and anaerobic digestion (AD), with a biomethane potential (BMP) between 301 – 365 mL/gVS. The kinetics of the AD showed rapid degradation, allowing for an efficient biogas process. Without additive, SBL can be adequately ensiled in vertical columns at ambient temperature thereby enabling the silage to be pumped directly into anaerobic digesters. Volatile solids of the silages increased towards the bottom of the column. The BMPs also increased towards the bottom of the column and differed by more than 20 % between the sampling points at 0.20 and 3.40 m. Except for silages at the top of the column, SBL biodegradability of 68 % was obtained, which is comparable to the biodegradability of the silages that were stored in bags. Gravity effect ensured that the silages below the top of the column were compressed, leading to a reduction in pore volume, improved quality of ensiling and higher methane yield compared to the silages from the bags. SBL harvesting method did not significantly affect the BMP. Although the addition of inoculants during ensiling resulted in an 8 % increase in BMP, a trade-off must be made between the cost of the additive and the gain from the extra BMP. The AD process of ensiled sugar beet leaves can be adequately described by the one-step-two-fractions kinetic model and can therefore be used to optimize process parameters for enhanced biomethane production.

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