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Anaerobic Biodegradability of Digestates – Influence of and Correlations for Klason lignin

Appropriate evaluation of the process performance of biogas plants needs to consider the anaerobic biodegradability of the used biomass. Anaerobic biodegradability is limited by lignin, which is part in most substrates and, by extension, in digestates of biogas plants. Previous research has shown that the content of acid detergent lignin (ADL) in digestates can be predicted from measured gross calorific values (GCVs). The correlation of GCV of 34 digestate samples to an alternative measure for lignin, the Klason lignin (KL), is evaluated as well as the correlation of KL content and other chemical constituents to residual biomethane potential (BMP). Results indicate a very low correlation of chemical composition to GCV and BMP. A correlation of GCV to BMP was not observable. The results let conclude that evaluation of anaerobic biodegradability of digestates by measuring KL or predicting KL from GCV is not productive.

Keywords: Anaerobic biodegradability, Biogas, Biomethane potential, Calorimetry, Klason lignin

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1 Introduction

In Germany in 2017, biogas plants produced 7.8 % of the electrical energy and have a significant impact to the energy production [1]. Most biogas plants use energy crops and residues from the agricultural sector (e.g., manure) for biogas production.

The energy efficiency of commercial-scale biogas plants is of high interest for the owners due to increasing substrate and operational costs. Traditionally, evaluating the efficiency of power plants is performed by energy balances using the gross calorific value (GCV) of the material streams as measure for the energy content. This method seems also be applicable for biogas plants with slight adaptations [2]. As basic parameters, the dry matter (DM) and volatile solids (VS) contents describe the water and organic content of the materials. Today most samples were investigated for these contents but the DM and VS do not allow any statement about the biodegradability, hence it is a sum-parameter.

For efficiency evaluation of biogas plants, the biodegradability of organic matter under anaerobic conditions has to be taken into account, so that the residual energy potential in the digestate is not overestimated. Anaerobic biodegradability of organic material is typically assessed using biomethane potential tests. As these tests are costly, time-consuming, and depend on several influencing factors (e.g., inoculum, substrate, temperature, inhibitors, trace element availability etc.) [3–5], there is a need for easier methods to predict anaerobic biodegradability. Studies examining the relation between biomethane potential and chemical composition of biomass have demonstrated

that from the analyzed components the lignin content, determined as acid detergent lignin by the Van Soest method, affects biodegradability the most [6–9].

Lignin has a negative impact on biodegradability and thus biomethane potential, as it is non-degradable under anaerobic conditions and inhibits degradation of hemicellulose and cellulose due to their incrustation. For determination of the lignin content different procedures are available which can be divided into gravimetric and spectrophotometric methods. The most common procedure in forage analytics is the acid-detergent lignin procedure, based on the method of Van-Soest. The second gravimetric method is from Klason. Other approaches like acetyl-bromide-lignin or thioglycolic acid-lignin belong to the spectrophotometric methods [10]. Gravimetric methods are based on complete hydrolysis and solubilization of the carbohydrates. The lignin is recovered in a solid fraction and can be measured directly. The spectrophotometric methods are based

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on complete solubilization of the lignin with acids. The lignin is determined indirectly with an spectrophotometer, which has to be calibrated with pure lignin.

Our previous research has shown that the content of acid detergent lignin (ADL) in digestates and mixtures of energy crops and manure correlates quite strongly ($R^2 = 0.89$) with the volatile solids specific *GCV* of these materials [11]. Therefore, prediction of the residual non-degradable energy content is possible using only the *GCV*, which is already applied for energy balancing.

However, the accuracy of prediction needs to be improved. Studies in forage analytics have demonstrated that the lignin content is underestimated using ADL methodology and that Klason lignin (KL) gives higher values and better results for recalculation of energy contents [12, 13].

Therefore, the aim of this study is to investigate correlations between the content of KL instead of ADL, gross calorific value, and residual biomethane potential in order to predict the anaerobically non-degradable fraction in digestates. For the investigation fermentation residues were used since there is less influence expected by components like proteins and lipids.

2 Material and Methods

2.1 Experimental Design

All samples were collected to investigate correlations of different analytical parameters in digestates. The samples were analyzed for different parameters: residual biomethane potential (*BMP*), content of dry matter and organic dry matter, water- and ethanol-soluble extractives, Klason lignin, acid-soluble lignin, hydrolyzed sugars, carbon content, and *GCV*. The analytical results were examined for correlations between each other.

2.2 Sample Origin

In total 34 digestate samples were collected from 32 full-scale agricultural biogas plants located in Germany. Samples 6 and 9 originate from the same biogas plant. Sample 6 is a liquid digestate of a digestate storage tank. Sample 9 is the dried solid phase of the same material like sample 6. Also samples 33 and 34 originate from the same biogas plant, but from different digesters in parallel operation. The criteria used for selection of sampled biogas plants were: different hydraulic retention times and commonly used input materials from the agricultural sector.

An overview of the biogas plants and their characteristics is given in Tab. 1.

2.3 Calculation of Hydraulic Retention Time

All biogas plant digesters were continuous stirred-tank reactors. The past hydraulic retention time of the samples was calculated by dividing the total digester volume of the respective biogas plant by the input material stream to the plant. The input material streams were recorded as mass. Conversion of mass to volume was conducted assuming a mass density of

1000 kg m^{-3} . For grains the mass density was analyzed as 1300 kg m^{-3} and used for calculation of the volume.

2.4 Analytical Procedure

The fresh digestate samples were analyzed for *BMP* at 37°C , for 60 d according to VDI 4630 [3] using a Hohenheimer biogas potential test equipment. Sample volume was 50 mL. The liquid digestate samples were treated like inocula without addition of a substrate and without dilution. The solid digestate sample (Sample No. 9) was considered like a substrate with the addition of inoculum. The ratio of VS of substrate to inoculum was 0.5. The inoculum for this sample was the liquid digestate of the respective biogas plant. The biomethane production from the inoculum was subtracted from the results in this case. Analyses were performed with three replicates.

For further analysis, samples were dried for conservation and analyzed for *DM* content according to EN 12880 [14], by oven-drying at 105°C for 24 h. Afterwards, the samples were grinded to approximately 0.5 mm. The content of VS was determined according to EN 12879 [15] by burning samples at 550°C for 4 h. The *GCV* was measured according to DIN EN ISO 18125 [16] in a bomb calorimeter.

The samples were analyzed in duplicate using a DIONEX ASE 200 extractor to determine the water- and ethanol-soluble extracts. The extraction was conducted with water, followed by ethanol with each three cycles at 100°C , 5 min heat time, 7 min static time, and a pressure of 10.34 MPa. The washing liquids were not further analyzed for extracted components. The extracted samples were further investigated to determine their lignin content.

The analysis for lignin was conducted according to the method from Klason [10]. In this procedure, some of the lignin is solved in the hydrolysate. Hence, the hydrolysate was analyzed for acid-soluble lignin via spectrophotometry at 205 nm wavelength. Acid-soluble lignin and Klason lignin were summed up for total lignin content (tKL). Additionally, the hydrolysate from the Klason procedure was examined for the content of hydrolyzed sugars from breakdown of hemicelluloses and cellulose via high-performance liquid chromatography. Samples 10, 13, 21, and 24 were not analyzed for hydrolyzed sugars.

2.5 Calculations and Statistical Methods

Calculations were carried out on VS-based parameters, except for extractives. As the content of extractives can be both, organic and inorganic, it is not suitable to rely the value to the content of VS. VS-based parameters were chosen because this is the interesting fraction in the digestate regarding residual biomethane and energy potential. For examining the connection between the *GCV*, the biomethane potential as well as the chemical composition of the digestates, simple and multiple linear regression models were applied.

Linear models seem to be reasonable because chemical characteristics like the *GCV* typically can be explained by linear combination of the single chemical constituents. By that, the chemical constituents would be the independent variables and

Table 1. Plant characteristics and input materials (in kg kg⁻¹).

Sample	Retention time [d]	Maize silage	Gras silage	Cereal grain	Cereal silage	Maize grain	Sugar beets	Cattle slurry	Pig slurry	Cattle manure	Poultry manure	Other
1	207	0.67		0.03	0.19		0.10					
2	234	0.65		0.03	0.21		0.11					
3	83	0.40	0.09		0.03			0.48				
4	100	0.42				0.02	0.14		0.41			
5	48	0.11	0.02	0.02			0.24	0.42		0.20		
6	96	0.65	0.10				0.25					
7	140	0.59							0.41			
8	202	0.34	0.08	0.02	0.05			0.52				
9	96	0.65	0.10				0.25					
10	120	0.75	0.15							0.09		
11	242	0.56	0.10							0.10	0.24	
12	55		0.15					0.57		0.23		0.05
13	132	0.39	0.01	0.01				0.54		0.05		
14	118	0.31	0.01	0.02				0.60		0.05		0.03
15		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	307	0.30	0.06	0.01	0.29			0.34				
17	140	0.63						0.32				
18	138	0.64	0.12	0.03	0.20							
19	112	0.66	0.11		0.11			0.13				
20	128	0.47	0.08		0.03	0.04		0.38				
21	94	0.39	0.09		0.13			0.39				
22	105	0.10	0.33			0.11		0.42		0.01		
23	141	0.40	0.38		0.18	0.04						
24	310		0.31	0.10	0.59							
25	242	0.24	0.50	0.05		0.21						
26		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	97	0.32	0.09	0.36	0.23							
28		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
29	280	0.76	0.18		0.04	0.00		0.02				
30	63	0.17	0.77	0.00							0.06	
31	211	0.03	0.61	0.03						0.33		
32	310	0.06	0.56	0.01	0.01	0.01				0.18	0.17	
33	116	0.02	0.65	0.01						0.28	0.04	
34	159	0.02	0.65	0.01						0.28	0.04	

the *GCV* and *BMP* would be dependent variables. But as the lignin content should be predicted, some models are inverted using the lignin fraction as dependent variable. The number of data points is reduced in some models due to missing values.

No data points were excluded as outliers. For simple and multiple linear regression models, regression coefficients were tested to be significantly different from zero using the *T*-test with a significance level of 0.05.

Also a partial least squares (PLS) regression model was calculated to find structures in the data and better correlations than in the simple linear regression models. Leave-one-out crossvalidation was employed. Statistical calculations were performed with the Software R, using the packages readxl, mda-tools, cowplot, and ggplot2 [17–21].

3 Results and Discussion

To find correlations of different measures for anaerobic biodegradability, the chemical composition, GCV, and residual

biomethane potential were analyzed. Analytical results for all 34 samples are summarized in Tab. 2.

As indicated in Tab. 2, the *DM* content varied between 42.38 g kg⁻¹ fresh matter (FM) and 917.82 g kg⁻¹ FM, where sample 9 with 917.82 g kg⁻¹ FM is a dried solid phase of the fermentation residue. Without the dried materials, the maximum is 175.7 g kg⁻¹ FM. The *VS* content of the samples varied between 506 g kg⁻¹ and 805 g kg⁻¹ *DM*. The observed *VS*-specific GCV was in the expected range of 20–24.7 MJ kg⁻¹ compared to previous studies [22]. For the extractives the observed range was between 235.1 and 463.4 g kg⁻¹ *DM*. Hydrolyzed sugars ranged between 148.63 and 314.13 g kg⁻¹ *VS*. The measured

Table 2. Sample characteristics (mean values + standard deviation) estimated from replicated measurements.

S.	<i>DM</i> _{FM} [g kg ⁻¹]	<i>VS</i> _{DM} [g kg ⁻¹]	<i>GCV</i> _{VS} [MJ kg ⁻¹]	<i>BMP</i> _{VS} [L kg ⁻¹]	<i>Ex</i> _{DM} [g kg ⁻¹]	<i>tKL</i> _{VS} [g kg ⁻¹]	<i>HS</i> _{VS} [g kg ⁻¹]	CC [ma %]
1	62.65±0.62	805.29±1.77	23.42±0.16	100±3	283.0±8.8	313.4±1.6	285.2±0.7	39.8±1.2
2	50.28±0.28	798.04±2.06	22.94±0.08	186±4	295.8±6.2	374.1±2.1	283.6±1.9	39.8±1.2
3	70.70±0.82	735.95±0.62	20.93±0.12	160±5	310.3±0.4	306.9±2.2	309.9±2.1	36.9±1.1
4	42.38±0.44	695.33±2.53	23.18±0.16	70±3	410.7±8.7	347.9±1.5	155.4±2.2	35.7±1.1
5	73.68±1.09	710.20±1.72	23.17±0.28	95±2	235.1±1.3	390.6±2.6	210.9±4.7	34.2±1.1
6	63.43±0.94	735.66±1.65	24.52±0.13	31±2	304.1±1.3	403.8±3.5	273.0±1.7	37.9±1.2
7	66.93±0.06	722.51±0.36	20.59±0.14	84±1	312.9±2.8	362.8±3.1	216.6±0.2	36.2±1.1
8	57.10±0.16	731.33±0.69	21.49±0.05	64±5	347.9±3.0	385.4±3.8	188.3±0.4	37.6±1.2
9	917.82±4.71	754.66±1.77	22.35±0.12	82±5	320.9±4.5	325.3±6.8	254.2±5.7	38.6±1.2
10	109.44±1.43	745.43±0.99	22.14±0.06	64±4	258.3±1.2	388.9±6.8	NA	36.1±1.1
11	111.48±0.50	726.28±2.62	21.69±0.11	68±1	253.4±4.5	333.1±1.3	314.1±4.3	35.1±1.1
12	74.20±0.71	697.39±1.34	23.92±0.11	60±1	379.1±2.1	368.7±1.7	176.4±0.5	35.1±1.1
13	81.79±0.46	762.05±4.16	22.54±0.16	210±8	266.4±2.0	337.0±4.0	NA	39.1±1.2
14	53.97±0.31	694.65±1.40	22.72±0.07	30±1	311.2±0.0	366.9±3.1	148.6±2.4	34.3±1.1
15	69.90±0.61	671.93±5.26	22.55±0.64	54±1	364.5±1.1	342.4±4.0	192.4±1.7	34.1±1.1
16	74.37±0.25	699.23±4.93	21.97±0.27	44±2	312.7±4.7	385.9±4.6	236.4±12.0	36.1±1.1
17	79.23±0.59	506.10±0.61	20.29±0.65	71±4	463.4±2.1	224.2±1.3	248.7±1.3	25.0±0.8
18	73.20±0.26	687.87±6.33	21.42±0.31	25±1	248.5±0.8	423.5±3.9	214.8±7.8	35.3±1.1
19	72.27±0.61	748.93±1.40	21.59±0.15	44±3	313.1±2.3	372.7±3.3	219.3±3.7	37.8±1.2
20	56.83±0.25	674.40±4.25	22.73±0.32	30±1	433.5±7.6	328.8±2.1	182.1±3.3	35.9±1.1
21	66.47±0.15	703.43±5.58	22.25±0.32	40±2	363.2±1.3	345.8±5.6	NA	36.4±1.1
22	96.93±0.42	698.23±0.59	21.98±0.22	57±1	413.7±2.0	325.8±2.7	189.5±3.5	34.8±1.1
23	79.93±0.35	679.87±1.65	22.34±0.17	58±1	411.3±3.0	320.9±9.0	221.1±3.6	35.6±1.1
24	70.37±1.70	720.83±4.22	20.71±0.57	45±3	289.0±1.4	353.5±2.7	NA	35.8±1.1
25	67.67±0.51	696.30±4.50	23.29±0.22	40±3	396.3±10.7	319.3±2.8	209.5±5.8	36.2±1.1
26	59.87±2.14	758.57±±7.42	22.17±0.37	21±1	289.5±2.5	356.5±3.7	299.4±6.0	37.8±1.2
27	76.83±1.25	745.20±±1.39	22.52±0.85	32±4	282.0±1.8	382.4±2.8	267.5±7.1	36.4±1.1
28	82.90±0.44	697.87±±7.41	22.21±0.51	29±1	315.6±3.2	362.3±5.5	266.5±6.0	34.7±1.1
29	81.18±0.49	770.45±±4.28	21.39±0.78	91±5	359.6±1.0	299.2±4.4	226.2±8.5	37.3±1.2
30	165.22±1.63	714.74±±6.22	20.45±0.66	90±7	329.7±0.3	303.6±7.0	285.1±7.7	35.7±1.1
31	92.59±0.26	716.48±±3.77	20.62±0.27	57±5	347.5±2.1	334.4±1.8	197.3±1.4	35.1±1.1
32	175.69±0.98	697.98±±1.13	20.02±0.05	70±4	297.3±1.6	323.1±2.5	255.1±0.8	34.2±1.1
33	123.50±0.71	692.7±±8±6.28	21.82±0.29	91±2	291.4±6.6	377.8±3.7	229.6±6.2	34.4±1.1
34	104.19±1.36	712.91±±4.44	22.19±0.47	112±7	308.6±6.2	363.4±3.4	256.9±1.7	35.5±1.1

Klason lignin accounted for 199.6–400.7 g kg⁻¹ VS. The acid-soluble lignin ranged from 18.4 to 34.7 g kg⁻¹ VS so the total lignin added up to 224.2–423.5 g kg⁻¹ VS.

However, extractives, hydrolyzed sugars, and tKL not summed up to the VS content for some samples. In these cases, not all organic components have been covered by the analytical procedure, e.g., hydrolyzed components other than sugars and acid-soluble lignin. If the calculated sum is above 100 %, there might be a part of the extractives related to inorganics, e.g., salts.

Fig. 1 demonstrates the correlation between GCV and the single chemical constituents. Correlations for hydrolyzed sugars and extractives were not significant. The GCV showed only a weak correlation with tKL. The fitted model equation allowed to calculate the GCV of pure lignin with 29.4 MJ kg⁻¹, which corresponds approximately to literature values [23–25]. The GCV of lignin-free organic matter can be calculated to be 17.9 MJ kg⁻¹, which is near to the GCV of polysaccharides with

17.3–18.6 MJ kg⁻¹ [23–25]. Therefore, the model equation principally follows the theoretical expectations that the GCV adds up linearly from the single constituents of tKL, hydrolyzed sugars, and extractives.

The unexplained variance in the dataset might originate from extracts or the different feed composition of the sampled biogas plants. It is known that the composition of lignin in plant fibers depends on the plant species [26]. So, the feed composition may also influence the GCV of pure lignin in the digestate samples. This was tested with lignin prediction models.

Models for the prediction of lignin, whereby lignin is the dependent variable here, are summarized in Tab. 3. All models show a relatively high error of prediction, as indicated by the error of crossvalidation *RMSE_{cv}*. In model No. 2, the GCV and interactions between the GCV and the single substrate components of the sampled biogas plants are used as predictors. However, also this does not improve prediction accuracy compared

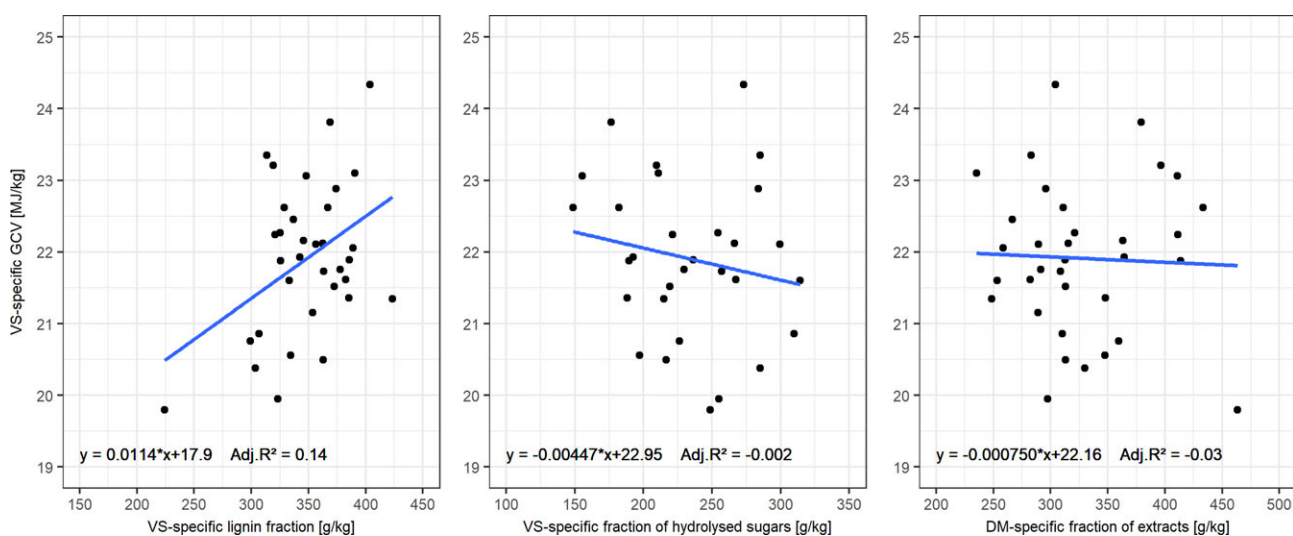


Figure 1. Linear regression models using GCV as dependent variable.

Table 3. Models for prediction of lignin in digestates.

Model No.	Model	N	Adjusted R ²	RMSE _c [g kg ⁻¹]	RMSE _{cv} [g kg ⁻¹]
5	$BMP = -1.85GCV + 111$	34	-0.03	43.65	45.24
6	$BMP = -0.246tKL + 157$	34	0.02	42.70	44.42
7	$BMP = 0.172Ex + 127$	34	0.02	42.62	44.29
8	$BMP = 0.307HS - 3.60$	30	0.10	35.36	37.81
9	$BMP = 0.318HS + 0.0177Ex - 11.9$	30	0.07	35.35	38.50
10	$BMP = 0.283HS - 0.161tKL + 57.9$	30	0.10	34.81	38.51
11	$BMP = 0.138HS - 0.353tKL - 0.195Ex + 223$	30	0.09	34.26	39.11
12	$BMP = 0.323HS - 0.228tKL - 0.118Ex - 0.0289HRT + 120$	27	0.14	32.29	40.13
13	$BMP = 0.758HS - 0.340tKL - 0.0697Ex + 649x_1 + 650x_2 + 494x_3 + 744x_4 + 733x_5 + 677x_6 + 706x_7 + 811x_8 + 817x_9 + 381x_{10} + 473x_{11} - 633$	27	0.15	23.74	64.54

x_1 = maize silage, x_2 = gras silage, x_3 = cereal grain, x_4 = cereal silage, x_5 = maize grain, x_6 = sugar beets, x_7 = cattle slurry, x_8 = pig slurry, x_9 = cattle manure, x_{10} = poultry manure, x_{11} = other, *HS* = hydrolyzed sugars, *Ex* = extractives, *tKL* = total Klason lignin, *BMP* = biomethane potential.

to model No. 1. So, the different plant species in the feed composition seem not to be the clue for reducing prediction errors.

The PLS model (model No. 4) using all analyzed parameters shows better correlation, but no obvious improvement in prediction accuracy compared to all other models. This PLS model provided the best performance using two components, where the content of VS and hydraulic retention time (*HRT*) had most impact. However, *HRT* exhibited no significant influence when using multiple linear regression models (data not shown).

The *RMSE_{cv}* of models No. 1–4 are comparable to a model that predicts acid detergent lignin from *GCV*s of digestates [11]. The lower R^2 values in the present study may originate from the lower spread in the values for tKL. In conclusion, the *GCV* seems not to be sensitive enough to predict tKL appropriately.

The correlation between the chemical composition and the residual biomethane potential is displayed in Fig. 2. Correlations for tKL and extracts are not significant. The correlation between *BMP* and hydrolyzed sugars is very low, but follows the principal expectation that higher sugar contents lead to higher *BMP*.

The models for prediction of the residual biomethane potential are summarized in Tab. 4. All examined models show low

R^2 values and high prediction errors. The *BMP* seems to be strongly influenced by uncovered factors, possibly by the particle size and structure of the organic material in the digestate, the used microbiology in *BMP* tests, and duration of the *BMP* test.

However, in literature no models could be found for prediction of *BMP* from digestates of biogas plants. Existing models are related to undigested lignocellulosic biomass [27]. Prediction of sample 2 and 13 by the model of Thomsen [27] leads to realistic values, all other samples were highly overestimated ($rRMSEP = 214\%$). Overestimation of the *BMP* could be related to the inaccessibility of cellulose and hemicellulose by lignin structures. Applying the model for undigested biomass could lead to more realistic values by higher available cellulose structures.

As correlations of chemical constituents to *BMP* and to *GCV* are very low, a correlation between *GCV* and *BMP* is non-existent (see Tabs. 3 and 4). The results let conclude that neither a prediction of *BMP* nor of the chemical composition regarding tKL is possible from simple *GCV* measurements. *GCV* alone is not sensitive enough for accurate prediction of anaerobic biodegradability. For rough prediction the correlation of *GCV* to ADL can be applied [11].

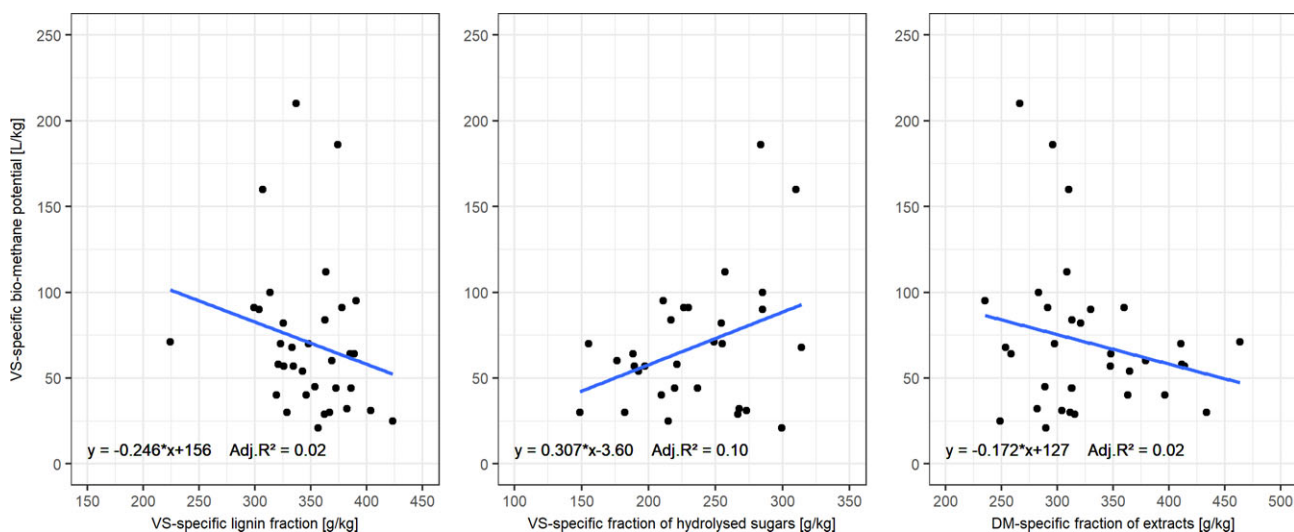


Figure 2. Linear regression models using biomethane potential as dependent variable.

Table 4. Models for prediction of residual biomethane potential in digestates.

Model No.	Model	<i>N</i>	Adjusted R^2	<i>RMSE_c</i> [g kg ⁻¹]	<i>RMSE_{cv}</i> [g kg ⁻¹]
1	$tKL = 14.4GCV + 33.0$	34	0.14	34.39	36.7
2	$tKL = GCV(-43.3 + 60.3x_1 + 59.2x_2 + 63.3x_3 + 62.3x_4 + 52.4x_5 + 56.4x_6 + 59.6x_7 + 62.2x_8 + 65.7x_9 + 56.5x_{10} + 29.9x_{11}) - 18.5$	30	0.13	27.92	46.06
3	$tKL = -0.183BMP + 361$	34	0.02	36.78	38.40
4	Including the parameters: substrate composition, <i>HRT</i> , <i>GCV</i> , <i>tKL</i> , <i>BMP</i> , <i>Ex</i> , <i>HS</i> , <i>DM</i> , <i>VS</i> , <i>CC</i>	27	0.48	29.33	35.47

4 Conclusion

In order to make a statement about biodegradability under anaerobic conditions, the analysis of residual methane potential still seems to be the most practical method. However, it does not allow a direct statement about possible potentials that still could be exploited. Here, chemical analysis seems to be more suitable. However, in this study, no good correlation between residual methane potential and the chemical composition could be found which may be related to the analytical method (Klason). In contrast, good correlations for the methane potential for substrate with the VanSoest method have been described in the literature.

The calorific value seems to allow a quick and rough estimation of the ADL content in fermentation residues. However, there is no appropriate correlation to the total Klason lignin content. The calorific value seems to be not sensitive enough for a more accurate estimation in this case. However, influencing effects like inaccessible carbohydrates may result in different KL values and should be analyzed in further research.

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Symbols used

BMP	[L kg ⁻¹]	biomethane potential
DM	[g kg ⁻¹]	dry matter
GCV	[MJ kg ⁻¹]	gross calorific value
HRT	[d]	hydraulic retention time
N	[-]	number of data points
RMSE _c	[g kg ⁻¹]	root mean squared error of calibration
RMSE _{cv}	[g kg ⁻¹]	root mean squared error of crossvalidation
rRMSEP	[g kg ⁻¹]	relative root mean squared error of prediction
t _{KL}	[g kg ⁻¹]	total Klason lignin
Ex _{DM}	[g kg ⁻¹]	extractables
HS _{VS}	[g kg ⁻¹]	hydrolyzed sugar
CC	[ma %]	carbon content

Abbreviations

ADL	acid detergent lignin
FM	fresh matter
KL	Klason lignin
VS	volatile solids

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