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A novel mathematical modelling of waste biomass decomposition to facilitate rapid methane potential prediction

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Abstract

Biogas production is known to be the most sustainable bioenergy technology available owing to its utilization of waste biomass. Due to the widely ranging energy contents of major organic carbons and their diverse degradation pathways, as well as their highly varying hydrolysis capacities, predicting methane yield is not as simple as predicting the production of other biofuels. This study investigated the hydrolysis behaviour of organic compounds using the operational conditions of real-scale biogas plants (hydraulic retention time (HRT) of 15, 20, 30, and 45 days) and explored a new approach to determine the biochemical methane potential (BMP), which can be used to predict the methane yield of a wide range of agro-industrial wastes. The degradation of hemicelluloses and cellulose increased gradually, closely following first-order degradation kinetics, at longer retention times. In spite of their longer retention times, only 45% of hemicelluloses and 34% of cellulose decomposed. The newly proposed BMP model was validated using 65 internal and external datasets. The model error (RMSE_p) was in the range of 37.4 to 87.7 NL CH₄ kg VS⁻¹ while the relative model error (rRMSE_p) was in the range of 12.1%–47.8%. The model fits best to gently lignified biomass, but the overestimated results obtained with woody biomass and winter harvested grass indicate that further investigation of the hydrolysis of well-lignified biomass is required to enhance the precision of the model.

Keywords: Anaerobic digestion; BMP model; lignocellulose; lignin; CSTR; methane
## Nomenclature

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldahl nitrogen</td>
</tr>
<tr>
<td>TAN</td>
<td>Total ammoniacal nitrogen</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral detergent fiber</td>
</tr>
<tr>
<td>ADF</td>
<td>Acid detergent fiber</td>
</tr>
<tr>
<td>ADL</td>
<td>Acid detergent lignin</td>
</tr>
<tr>
<td>BMP</td>
<td>Biochemical methane potential</td>
</tr>
<tr>
<td>TBMP</td>
<td>Theoretical biochemical methane potential</td>
</tr>
<tr>
<td>df_i (%)</td>
<td>The decay of an organic compound i</td>
</tr>
<tr>
<td>df_∞ (%)</td>
<td>The ultimate decay of an organic compound i</td>
</tr>
<tr>
<td>k (day⁻¹)</td>
<td>The decay rate constant for a fraction</td>
</tr>
<tr>
<td>θ (day)</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>rᵦᵣ (NL CH₄ L⁻¹ day⁻¹)</td>
<td>The specific rate of CH₄ production</td>
</tr>
<tr>
<td>Mᵢ₀ (NL CH₄ kg VS⁻¹)</td>
<td>TBMP of organic compounds (i) in the input feedstock</td>
</tr>
<tr>
<td>RMSEP</td>
<td>Residual mean square error in prediction</td>
</tr>
<tr>
<td>rRMSEP</td>
<td>Relative residual mean square error in prediction</td>
</tr>
<tr>
<td>VS ed</td>
<td>Easily degradable VS</td>
</tr>
<tr>
<td>VS sd</td>
<td>Slowly degradable VS</td>
</tr>
<tr>
<td>VS nd</td>
<td>Non-degradable VS</td>
</tr>
<tr>
<td>XP</td>
<td>Crude proteins</td>
</tr>
<tr>
<td>XL</td>
<td>Crude lipids</td>
</tr>
<tr>
<td>VS XP (%)</td>
<td>Concentration of crude protein in VS</td>
</tr>
<tr>
<td>df XP (%)</td>
<td>Ultimate degradation factor of VS XP</td>
</tr>
<tr>
<td>VS HM (%)</td>
<td>Concentration of hemicellulose in VS</td>
</tr>
<tr>
<td>df HM (%)</td>
<td>Ultimate degradation factor of VS HM</td>
</tr>
<tr>
<td>VS CE (%)</td>
<td>Concentration of cellulose in VS</td>
</tr>
<tr>
<td>df CE (%)</td>
<td>Ultimate degradation factor of VS CE</td>
</tr>
<tr>
<td>BMP_p (NL CH₄ kg VS⁻¹)</td>
<td>Predicted BMP</td>
</tr>
<tr>
<td>VS VFA (%)</td>
<td>Concentration of VFA in VS</td>
</tr>
<tr>
<td>VS EL (%)</td>
<td>Concentration of ethanol in VS</td>
</tr>
<tr>
<td>VS CL (%)</td>
<td>Concentration of crude lipid in VS</td>
</tr>
<tr>
<td>VS CB (%)</td>
<td>Concentration of non-fibrous carbohydrates in VS</td>
</tr>
<tr>
<td>TBMP VFA (NL CH₄ kg VS⁻¹)</td>
<td>Stoichiometric methane potential of VS VFA</td>
</tr>
</tbody>
</table>
1. Introduction

Biogas production by anaerobic digestion (AD) is known to be the most sustainable bioenergy technology available owing to its utilization of waste biomass as a substrate, greenhouse gas reduction efficacy, and production of digestate, which is used as a fertilizer (Saidu et al., 2013). The flexibility of using biomass as a source of energy is another benefit. For example, while bioethanol and biodiesel require specific types of biomass as substrates, biogas can be generated from any organic compound, either solid or liquid. Therefore, a wide range of biomasses, including animal slurry, agricultural residues, municipal solid waste, and food processing waste can be used to produce biogas (Tuesorn et al., 2013).

Owing to the diverse AD pathways of organic carbons, predicting the actual methane yield is not as simple as predicting the yield of other biofuels. Furthermore, owing to the flexibility in the biomasses that can be used for biogas production and the fact that AD of single substrates (mono-digestion) suffers from drawbacks related to substrate properties, co-digestion of different biomasses has been implemented in industrial biogas plants (Mata-Alvarez et al., 2014). The co-digestion of organic waste materials with animal manure is appealing for biogas production from food, food processing, slaughterhouse, fruit, and vegetable wastes (Llaneza Coalla et al., 2009). However, the full energy content of major organic carbons ranges widely from 375 \( \text{NL CH}_4 \text{ kg}^{-1} \) (organic acids) to 1014 \( \text{NL CH}_4 \text{ kg}^{-1} \) (lipid). Moreover, their hydrolysis behaviour, especially of lignocellulose-combined carbohydrates, is strongly affected by the physicochemical bonding within and between lignocellulosic macromolecules, i.e. the lignification degree, amorphous and crystalline contents in cellulose, and the degree of crystallinity are affected by the nature of bonding. Furthermore, the hydrolysis mechanism of these biopolymers and their structural
barriers against enzymatic hydrolysis are not clearly understood, which in turn hinders the
tensive use of alternative potential biomasses (Wilson, 2011).

Many researchers have attempted to derive the kinetic constants of specific biomasses to predict
biogas yield in real scale biogas reactors using the data from batch AD experiments (Ibrahim,
2014; Syaichurrozi et al., 2015). However, the decomposition environment in real scale
continuous biogas reactors is not similar to that in a batch test. Differences exist in terms of the
limited microbial population and their acclimation issues, agitation effect, etc. With regard to the
energy contents, biochemical methane potential (BMP) is the most commonly used parameter to
assess the methane potential of a biomass and numerous studies have dealt with analysing the
BMPs of a wide range of biomasses. Due to the time-consuming and labour-intensive
determination procedure as well as the high possibility of errors in the reproducibility of BMP
results, many researchers have suggested alternative measurements (Bayard et al., 2018; Bekiaris
et al., 2015; Cu et al., 2015; Doublet et al., 2013; Raju et al., 2011; Strömberg et al., 2015; Triolo
et al., 2014, 2011; Xu et al., 2014). Simple or multiple regression statistical approaches were
developed (Bayard et al., 2016; Cu et al., 2015; Kafle and Chen, 2016; Thomsen et al., 2014) and
spectroscopic methods, such as applying near infrared spectroscopy (Doublet et al., 2013; Raju et
al., 2011; Triolo et al., 2014) or Fourier transform mid-infrared photoacoustic spectroscopy
(Bekiaris et al., 2015), were suggested. However, the state-of-the art technology still requires
sample drying prior to obtaining the spectra, which makes real time analysis of BMP impossible.

The aim of the present study is to provide a new insight on the hydrolysis behaviour of major
organic compounds during co-digestion in the semi-continuous mode and to attempt at
developing a new BMP model from the results.

2. Materials and methods

2.1. Feedstock and inoculum
Energy-dense and easily degradable agro-industrial wastes and industrial wastewater as co-substrates (25 wt.%) and pig manure (75 wt.%) as prime feedstock were examined in both batch and semi-continuous modes. The co-digestion parameters, i.e. the mixing ratio and choice of co-substrate, were similar to those used commonly in Danish central biogas plants; in these plants, 25 wt.% of diverse energy-dense wastes (slaughterhouse waste, expired canned ham, fish and dairy waste, etc.) are typically co-digested with animal manure. Brewery wastewater was collected from a local brewery (Odense, Denmark) and processed meat (canned pork and ham) were purchased from supermarkets. Inoculum, pig manure and slaughterhouse waste were obtained from the Fangel biogas plant (Odense, Denmark). The feed for AD consisted of 75 wt.% pig manure and 25 wt.% of the co-substrate mixture. The final composition of the feed was 75 wt.% pig manure, 11.25 wt.% processed meat waste, 11.25 wt.% brewery waste, and 2.5 wt.% slaughterhouse waste. The dry matter (DM) and VS content of inoculum were 47.8 (±2.3) and 29.6 (±1.8) g kg\(^{-1}\) respectively.

2.2. Anaerobic digestion in the semi-continuous mode

Four stirred tank reactors (CSTRs) with a total volume of 20 L were utilized for the AD process. Each reactor was made of two stainless steel plates and an acrylic cylinder (diameter 360 mm and height 360 mm) was fixed between the two plates. The engine was installed at the bottom of the reactor to mix the medium using two radial flow-impellers of 0.2 m diameter. In order to supply the heat required for the reaction, four 50 W elements were attached to the bottom plate and were controlled by a temperature sensor inside the reactor. The gas exhaust and feeding inlet were supported by the top plate, while the two digestate outlets were fixed on the bottom plate at two different levels.

The inoculum was obtained from the Fangel biogas plant, which processes 75 wt.% of animal manure as the prime feedstock and 25 wt.% of industrial food processing waste at mesophilic conditions. After an acclimation period of 14 days, the four reactors were subjected to different organic loading rates (OLRs) and hydraulic retention times (HRTs) of 15, 20, 30, and 45 days.
under mesophilic conditions at 37°C. The volatile solid (VS) content of the feed mixture was
58.26 g kg\(^{-1}\) while the OLR values were 3.88 g VS L\(^{-1}\) day\(^{-1}\), 2.91 g VS L\(^{-1}\) day\(^{-1}\), 1.94 g VS L\(^{-1}\) day\(^{-1}\), and 1.29 g VS L\(^{-1}\) day\(^{-1}\). The reactors were fed on a daily basis and the gas production was monitored. Volatile fatty acids (VFAs) were measured twice a week to monitor the stability of the anaerobic digestion process. The AD operation was continued for a period of three times as long as the HRTs. At the end of the operation time, the digestion was terminated and the digestates were removed and placed in a freezer for further analysis.

2.3. BMP assay

The German standard method (VDI 4630, 2006) was used to determine the BMP of the substrates and digestates from each reactor using batch reactors of 1 L capacity. The inoculum-to-substrate ratio (I/S ratio) was set at 2.5 based on the VS content. All the experiments were conducted in triplicate. The biogas yield of inoculum as a blank test for the correction of gas production and methane yield of microcrystalline cellulose (Avicel® PH-101, Sigma-Aldrich) as a reference material was determined. After mixing the substrate and inoculum, which had already been degassed for two weeks, 150 mL of a nitrogen-flushed buffer medium was added to the inoculum and substrate mixture according to the ISO standard method. The VS of BMP tests was 4.7 % (<10 %) (ISO, 1995; Pham et al., 2013; Holliger et al., 2016). The batch digesters were flushed with nitrogen gas to provide a fully anaerobic system. The BMP test was performed under mesophilic conditions at 37°C for 60 days. The digesters were shaken manually to prevent layering and to boost degassing. A syringe (Hamilton Super Syringe) was used to measure the wet biogas volume and later, these gas volumes were corrected to dry biogas as normal litres. CH\(_4\) concentration was determined using a gas chromatograph (GC; 7890A, Agilent Technologies, USA) equipped with a thermal conductivity detector and a 30 m x 0.320 mm column (J&W 113-4332, Agilent Technologies, USA).

2.4. Sample and digestate analysis
The DM was measured according to a standard procedure (APHA, 2005) by drying the samples at 105°C. The ash content of the dry matter was determined after placing the dried samples in a muffle furnace at 550°C for 2 h and the VS content was calculated by the difference in the DM and ash content. Total Kjeldahl nitrogen (TKN) and total ammoniacal nitrogen (TAN) were determined according to a standard method (APHA, 2005). The crude lipid content was measured by Soxhlet extraction.

The obtained DM results were corrected because during DM measurement, easily volatile compounds evaporate. It is important to measure their concentration in the untreated sample and add this concentration to the oven-dried samples to obtain the corrected DM (Weissbach and Strubelt, 2008). VFA concentration from C$_2$–C$_5$ was determined using a gas chromatograph (Hewlett Packard 6890, Italy) with a flame ionization detector and a 30 m x 0.25 mm x 0.25 μm column (HP-INNOWax, USA) (Larsen et al., 2017). Ethanol content was measured by high-performance liquid chromatography (HPLC; Agilent 1100, Germany). For alcohol and VFA analysis, the samples were filtered through a 0.2 μm nylon membrane filter prior to injection into the GC and HPLC columns. For VFA determination, the pH of the samples was adjusted to about 2 by adding phosphoric acid (Larsen et al., 2017). Lignin, cellulose, and hemicellulose were determined according to Van Soest’s characterization for fibre analysis (Van Soest, 1963).

2.5. Data analysis

Crude protein was determined by multiplying the difference between TKN and TAN by 6.25 (Triolo et al., 2011). α-NDF was used to measure the total fibrous fractions consisting of hemicellulose, cellulose, and lignin. Acid detergent fibre (ADF) includes cellulose and lignin whereas acid detergent lignin (ADL) represents the lignin content. The hemicellulose content was calculated from the difference between α-NDF and ADF and cellulose content was calculated from the difference between ADF and ADL (Triolo et al., 2011).

Bushwell’s formula was used to calculate the theoretical biochemical methane potential (TBMP) of a specific compound with defined molecular formulae under standard conditions (273 K and
1 101.325 kPa). TBMP is a term estimated by assuming the total conversion of a specific
2 compound to methane and carbon dioxide (Symons and Buswell, 1933; Raposo et al., 2012):
3 \[ C_nH_aO_bN_C + \left( n - \frac{a}{2} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left( \frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left( \frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \] (1)
4 \[ B_{th} = \left( \frac{n/2 + a/8 - b/4 - 3c/8}{12n + a + 16b + 14c}\right)22400 \text{ NL CH}_4 \text{ kg VS}^{-1} \] (2)
5 TBMP of the substrate and digestate was calculated using the TBMP of each VS compound; the
6 TBMPs of VFAs, ethanol, crude proteins, crude lipids, lignin, hemicellulose, cellulose, and
7 carbohydrates were 373, 730, 496, 1014, 727, 415, 415 and 415 NL CH\(_4\) kg VS\(^{-1}\), respectively.
8
9 2.6. Degradation kinetics and CH\(_4\) production model
10 The hydrolysis of organic matter for biogas production is often described by first-order kinetics
11 (Angelidaki et al., 2009; Dandikas et al., 2018; Ebrahim-Nik et al., 2018; Hashimoto, 1989;
12 Triolo et al., 2014). As the degradation rate of organic compounds controlled by hydrolysis, first-
13 order kinetics is used to derive the kinetics equation. However consideration should be given to
14 the fact that each compound is composed of both degradable and non-degradable fractions.
15 \[ S = S_D + S_{ND} \] (3)
16 \[ \frac{dS_D}{dt} = -kS_D \] (4)
17 Therefore:
18 \[ S_D = S_{0D}e^{-k\theta} \] (5)
19 \[ df_\infty = \frac{S_{0D}}{S_0} \text{ and/or } df_\theta = \frac{S_{0D} - S_D}{S_0} \] (6)
20 From the combination of Eq. (5) and (6), the degradation kinetics would be as follows:
21 \[ df_\theta = df_\infty * (1 - e^{-k\theta}) \] (7)
where $S$ is the total concentration of an organic compound, $S_D$ is the concentration of the degradable part of an organic compound, $S_{ND}$ is the concentration of the non-degradable (inert) part of an organic compound, $df$ is the decay of an organic compound ($\%$) at a certain time, $df_\infty$ is the ultimate decay of an organic compound ($\%$), $k$ is the decay rate constant for an organic compound (day$^{-1}$) and $\theta$ is the HRT (days).

From Eq. (7), the specific rate of CH$_4$ production can be predicted as shown below:

$$r_{CH_4} = \frac{\sum_{i=1}^{n}(df_\theta \cdot M_i_0)}{\theta}$$

(8)

Here, $r_{CH_4}$ is the specific rate of CH$_4$ production (NL CH$_4$ L$^{-1}$ day$^{-1}$), $df_\theta$ is the decay of an organic compound $i$ ($\%$), and $M_i_0$ is the TBMP of the organic compounds $i$ in the input feedstock (NL CH$_4$ kg wet weight (ww$^{-1}$)).

During the validation of the BMP prediction model, the precision of the model was tested using external datasets by applying the statistical parameters of residual mean square error in prediction (RMSEP) and relative residual mean square error in prediction (rRMSEP), which is normalized RMSEP using the mean value of the measured BMP. Least-squares non-linear regression was performed by minimising the residual sum of squares (RSS):

$$\text{residual sum of square (RSS)} = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

(9)

where $\hat{y}_i$ is the predicted value and $y_i$ the measured value.

3. Results and discussion

3.1. Characteristics of the substrates and digestates

The physicochemical characterization results together with the concentrations of major organic compounds are presented in Table 1. Lignocelluloses were not present in any of the co-substrates, but in contrast, the pig manure was mainly composed of lignocellulose. The brewery wastewater was diluted to 7.8 g DM kg$^{-1}$ and was mostly composed of ethanol. The
slaughterhouse waste and processed meat waste, which were two types of semi-solid wastes, contributed to a concentration of 70 g DM kg$^{-1}$ in the feed mixture. The processed meat waste was rich in both proteins and lipids while the slaughterhouse waste contained mostly lipids. (Table 1 near here)

The characteristics of the pig manure used in this study (DM content and organic composition) were similar to those used in previous studies (Møller et al., 2004; Tsapekos et al., 2017). The high content of lignocelluloses in the pig manure clearly indicates that the pig manure is only a recalcitrant substrate in the feed mixture. Although plant biomass, typically wood or agricultural residues, such as straw and bagasse, are generally known as lignocellulosic biomasses, the lignocellulosic characteristics of animal manure have been pointed out in recent studies due to its large concentration (Bruni et al., 2010; Cu et al., 2015).

The organic composition of the mixture of these four substrates resulted in a fairly good balance of lipids, proteins, and lignocelluloses with a small content of dissolved VS sources, i.e. ethanol, VFAs, etc., which reduces the concentration of the recalcitrant lignocelluloses; meanwhile, the proteins and lipids became the dominant organic compounds in the feed mixture (Table 1).

### 3.2. Methane potentials

Table 1 shows that the organic compositions of the four substrates agree well with the BMP and biodegradability results. The BMP of pig manure was considerably lower than that of other co-substrates. In fact, the BMP of slaughterhouse waste, which mostly contains lipids, was approximately three times higher than the BMP of pig manure. The BMP calculated for pig manure is in good agreement with previously reported values (Triolo et al., 2013). The differences in the BMPs of the substrates were considerably more evident when the specific methane potential was considered (NL CH$_4$ kg ww$^{-1}$). The values of the specific methane potential of the substrates were 9 NL CH$_4$ kg ww$^{-1}$ (pig manure), 145 NL CH$_4$ kg ww$^{-1}$
(processed meat waste), 258 NL CH$_4$ kg ww$^{-1}$ (slaughterhouse waste), and 3.6 NL CH$_4$ kg ww$^{-1}$ (brewery wastewater).

Such large differences in the specific methane potentials of the substrates consequently led to diverse levels of contribution to the overall methane potential of the feed mixture. Among others, pig manure, which was 75 wt.% of the feed mixture, contributed to only 22% of the overall methane potential. Moreover, the brewery wastewater contributed to only 1% of the methane potential of the feed mixture. On the other hand, despite its content being only 2.5 wt.% in the feed mixture, slaughterhouse waste contributed to 23% of the overall methane potential due to its high solid and lipid concentration. In the feed mixture, the contribution of VFAs, ethanol, XP, XL, hemicellulose, cellulose, lignin and carbohydrate to its TBMP was 5, 1, 26, 47, 4, 6, 7 and 4 % respectively. Therefore, a large fraction of methane sources in the feed mixture consisted of lipids.

The results highlight that the contribution of brewery wastewater to the overall methane potential during the co-digestion of different biomasses was nearly negligible; however, it would be effective as a buffer medium to dilute so-called inhibitors, especially when AD is carried out with other protein and lipid substrates.

### 3.3. CH$_4$ production and CSTR performance

Specific biogas and CH$_4$ production rate (NL CH$_4$ L$^{-1}$ day$^{-1}$) and VFAs concentration during AD in semi-continuous mode in each reactor are plotted against time in Figure 1. It can be seen that the CH$_4$ production was steady and there was no clear inhibition in the gas yield. The CH$_4$ content in the biogas was very stable and varied from 69% to 71%. The high CH$_4$ concentration in the exhausted biogas was probably due to the presence of lipids in the feed.
The VFA concentration gradually diminished at greater HRTs: \((7.69 \pm 0.17) \text{ g kg}^{-1}\) (HRT 15 days), \((2.96 \pm 0.09) \text{ g kg}^{-1}\) (HRT 20 days), and \((0.65 \pm 0.05) \text{ g kg}^{-1}\) (HRT 30 days).

Accumulation of VFAs in the reactor was observed clearly at a HRT of 15 days; however, the considerably high level of VFAs was stable throughout HRT durations of 20 days and 30 days.

Aguilar et al. (1995) showed that there was no inhibition in acetate degradation when the VFA concentration was less than \(4 \text{ g L}^{-1}\); when it exceeded \(10 \text{ g L}^{-1}\), the AD process was inhibited (Aguilar et al., 1995). Fortela et al. (2016) found that activated sludge microbial consortia were highly inhibited at total VFA concentrations of \(10 \text{ g L}^{-1}\) and \(20 \text{ g L}^{-1}\) (Fortela et al., 2016).

TAN was moderately high, ranging from \(3.77 \text{ g kg}^{-1}\) to \(4.02 \text{ g kg}^{-1}\). Despite the high TAN content in the reactors, there was no clear inhibition of ammonia; for example, the VFA concentration varied widely with variations in the HRT and in the reactor with an HRT of 45 days, it was nearly zero in the steady state phase with no evidence of the inhibited methanogenic activity caused by ammonia. The unclear inhibition due to TAN can be attributed to the fact that the inoculum had adapted well after it was obtained from the biogas plant operated using manure, food processing waste, and slaughterhouse waste.

Previous studies on ammonia inhibition reported a wide range of ammonia inhibition activities (mostly expressed as TAN concentration) from \(1.7 \text{ g L}^{-1}\) to \(14 \text{ g L}^{-1}\); the large variations in the TAN level have been discussed in terms of the different substrates used, inocula, pH, temperature, other operational conditions, and acclimation (Chen et al., 2008; Yenigün and Demirel, 2013). Regarding the adaptation of methanogens and acclimation to ammonia, Yenigün and Demirel (2013) reported that AD occurred efficiently at concentration of TAN up to \(9 \text{ g L}^{-1}\) (Yenigün and Demirel, 2013).

### 3.4. Anaerobic digestion of organic compounds

The contents of crude lipids, crude proteins, cellulose, hemicelluloses, lignin, VFAs, and ethanol were measured before and after digestion (Figure 2). As expected, ethanol was completely
decomposed in all the reactors. It can be observed in Figure 2 that the degradation of lipids and
proteins was similar in all the reactors; it is noteworthy that crude lipid was not present in the
digestate in any of the reactors; 27% of the feed protein was present in the digestate, which
corresponds to a decomposition of 73% ± 3% of the crude protein. The remaining organic
nitrogen is probably non-degradable, i.e. lignin-combined nitrogen, or it is transformed to
microbial communities.

(Figure 2 near here)

Due to the presence of relatively high levels of VFAs at shorter HRTs, it is not clear whether the
lipid was completely digested or not within 15 days of AD; however, it can still be confirmed
that the entire lipid content was hydrolysed within 15 days and in addition, it can be stated that
the extent of hydrolysis of the lipid and protein was not affected by the operational time, given
that the retention time is long enough (≥15 days). It can be inferred from the biodegradability
index (BMP/TBMP) in Table 1 that the results obtained on the hydrolysis of lipids and proteins
were in good agreement with the ratio between BMP and TBMP. Consequently, the BMP of
lipid- and protein-rich biomass can be analyzed by measuring the protein and lipid content.

On the other hand, the degradation of the two lignocelluloses (hemicelluloses and cellulose)
increased gradually, closely following first-order degradation kinetics. Furthermore, the
degradation of hemicellulose was faster than that of cellulose. Interestingly, in spite of a long
retention time (HRT of 45 days), only 45% of the hemicelluloses and 34% of cellulose
decomposed, implying that 55% and 66% of hemicelluloses and cellulose, respectively, were
available in the feed mixture as compared to the initial feed.

The reason for the relatively easier hydrolysis of hemicellulose as compared to cellulose is due to
its amorphous structure. It is known that lignin does not degrade in the absence of oxygen and
our results also show that its concentration in all the digestates is nearly the same as its
concentration in the input feed mixture.
Table 3 presents the first-order decay kinetic constants of cellulose and hemicellulose and their statistical parameters calculated using Eq. (7). Figure 3 visualizes the degradation of cellulose and hemicellulose in terms of the measured and predicted values and the simulation results of 200 days extended retention time.

Residual analysis using Eq. (7) with 4 dataset points for both hemicellulose and cellulose indicates that hemicellulose was composed of 59.3% VS_{SD} while the remaining 40.7% was inert (non-degradable VS (VS_{ND})). In the case of cellulose, the fraction of VS_{SD} was even smaller at 36.3% (Figure 3). Furthermore, when the first-order decay kinetics model was fitted using the lignocellulose data points, it was found that 72.6% of the mass corresponded to VS_{ND}. Therefore, it can be roughly assessed that the biodegradability of lignocellulose was ~37.4%. This result is probably comparable with the biodegradability data of 55 lignocellulosic biomass samples reported previously by Triolo et al. (2012), who reported the following values: hedge cuttings, 39.9(7.6)%; woody cuttings 32.7(5.2)%; wild plants 44.9(12.5)% (Triolo et al., 2012).

3.5. Prediction of BMP

With the aim of providing a new and alternative method for analysing the BMP, we categorized organic compounds into three pools: easily degradable VS (VS_{ED}), VS_{SD}, and VS_{ND}. VS_{ED} includes non-lignocellulosic carbohydrate, organic acids, alcohols including all the intermediate products produced during hydrolysis, and methanogens; crude lipid was also categorized as VS_{ED} due to its complete decomposition within 15 days. Crude protein was divided into VS_{ED} and VS_{ND}, taking the remaining protein in the digestate into account. Hemicellulose and cellulose were defined as VS_{SD} and lignin VS_{ND}.

\[ VS_{XP} = df_{XP} VS_{XP} + (1 - df_{XP}) VS_{XP} \]  \hspace{1cm} (10)
Here, $VS_{XP}$ is the concentration of crude protein in VS and $df_{XP}$ is the ultimate degradation factor of $VS_{XP}$, which has a value of 0.73.

Eq. (11) and (12) were deduced from Table (3).

$$VS_{HM} = df_{HM} VS_{HM} + (1 - df_{HM}) VS_{HM}$$

$VS_{HM}$ is the concentration of hemicellulose in VS and $df_{HM}$ is the ultimate degradation factor of $VS_{HM}$, which has a value of 0.59.

$$VS_{CE} = df_{CE} VS_{CE} + (1 - df_{CE}) VS_{CE}$$

$VS_{CE}$ is the concentration of cellulose in VS and $df_{CE}$ is the ultimate degradation factor of $VS_{CE}$, which has a value of 0.36.

Finally, the BMP of the biomass can be estimated by Eq. (13).

$$BMP_P = TBMP_{VFA} VS_{VFA} + TBMP_{EL} VS_{EL} + df_{XP} TBMP_{XP} VS_{XP} + TBMP_{XL} VS_{XL} + df_{HM} TBMP_{HM} VS_{HM} + df_{CL} TBMP_{CL} VS_{CL} + TBMP_{CB} VS_{CB}$$

$BMP_P$ refers to the predicted BMP (NL CH$_4$ kg VS$^{-1}$); $VS_{VFA}$, $VS_{EL}$, $VS_{XP}$, $VS_{XL}$, $VS_{HM}$, $VS_{CL}$, and $VS_{CB}$ are VS compounds referring to VFA, ethanol, crude protein, crude lipid, hemicellulose, cellulose, and non-fibrous carbohydrates, respectively (kg kg VS$^{-1}$). $TBMP_{VFA}$, $TBMP_{EL}$, $TBMP_{XP}$, $TBMP_{XL}$, $TBMP_{HM}$, $TBMP_{CL}$, and $TBMP_{CB}$ are stoichiometric methane potentials (NL CH$_4$ kg VS$^{-1}$) corresponding to $VS_{VFA}$, $VS_{EL}$, $VS_{XP}$, $VS_{XL}$, $VS_{HM}$, $VS_{CL}$, and $VS_{CB}$, respectively; $df_{XP}$, $df_{HM}$, and $df_{CL}$ are the ultimate degradation factors of $VS_{XP}$, $VS_{XP}$, and $VS_{XP}$, respectively.

Different studies have used various approaches to predict BMP. These have mostly focused on a statistical model using regression and correlation between BMP and some variables, especially lignin, cellulose and hemicellulose (Bayard et al., 2016; Kafle and Chen, 2016; Thomsen et al., 2014; Triolo et al., 2011; Xu et al., 2014). In the BMP model suggested in this paper, the
coefficient for each compound was derived from their degradation kinetics, in which only
degradable components were taken into consideration. However further study is needed to
include the lignin effect on the degradation kinetics of cellulose and hemicellulose. As most of
the components were included in the present model, it can be used for a wide variety of
biomasses for which models based on the lignocellulosic fraction alone might not be very
effective or useful for proteinous and fatty substrates.

3.6. BMP model validation

The precision of the suggested model in predicting the BMP was tested using the samples from
this study. External validation was performed using the BMP results and compositions published
elsewhere as the reference data variables. The results obtained using the internal data were quite
precise with very low model error. The model error in prediction (RMSE$_{PE}$) was only 25 (NL CH$_4$
kg VS $^{-1}$); thus, the normalized model error was low as well (4.2% of rRMSE). Even if the BMP
prediction model was not built by directly using the BMP values from the testing set, the
satisfactory validation results would however still obtained because the same samples were used
for validation. Nonetheless, the very high precision seen in Table 4 is most interesting, which
indicates that the calibrated model exhibits high precision.

For the validation of the suggested model, external data from 10 maize samples (Oslaj et al., 2010)
from Oslaj et al. (2010) and 51 lignocellulosic samples from the authors’ previous study (Triolo et
al., 2012) were used as the testing sets. In total, 65 external data sets were tested. The
lignocellulosic data sets were divided into two categories: 1) grasses and agricultural residues and
2) woody samples, and they were tested separately. In the case of the grass and agricultural residues,
the model was tested with and without four well-crystallized winter-harvested grasses with
critically low methane potentials. This was done to test the responses of the model to winter-
harvested grasses.

(Table 4 near here)
The results of the validation exercise were found to be very interesting as the model had a tendency to overestimate the BMP of woody biomass and winter-harvested grasses (Figure 4; Table 5). On the other hand, it exhibited relatively fine precision when tested on green grasses with maize. The obtained rRMSE\textsubscript{PE} values for maize and grasses were 12.1% and 15.8%, respectively, which corresponds to a model error of 37.4 (NL CH\textsubscript{4} kg VS\textsuperscript{–1}) for maize and 49.9 (NL CH\textsubscript{4} kg VS\textsuperscript{–1}) for straw. When the winter-harvested grasses were included, the relative model error increased to 19.6%. $R^2$ decreased to 0.47 when winter grasses were excluded (Table 5). The higher correlation level compared to the lower model precision when including winter harvested grasses is typically seen in wider ranges of independent variables when including samples with very low methane potentials (Triolo et al., 2011).

The BMP model was most precise in the case of the maize samples. This was probably because of the highly homogeneous characteristics of the maize samples, when compared to a wide range of lignocellulosic biomasses harvested through different seasons. Despite a low model error for maize, there was no correlation found, probably due to low variation of methane potentials included in the model validation. Except for the woody and winter-harvested grass samples, the predicted and measured BMP values are not clearly biased, as can be seen in Figure 4.

Only a few BMP prediction studies on organic compounds carried out external validation; therefore, a comparison was not possible to analyze the precision of the BMP model. Comparing this model to the other alternative methods used to predict BMP (for e.g. near infrared spectroscopy (NIR)), it was found that the results were almost equivalent (Bekiaris et al., 2015).

4. Conclusions

This study emphasized that the majority of lipids and proteins in agro-industrial waste are transformed into methane in modern industrial biogas plants (HRT ≥ 15 days). The methane yield obtained by the degradation of the remaining hemicelluloses and cellulose increases at longer...
retention times. The model in this paper includes both lignocellulosic and non-lignocellulosic compounds, therefore it can be applied to a wide range of biomasses where the regression models based on the lignocellulose fraction alone might not be applicable for non-lignocellulosic biomasses. The developed BMP model fits best to lightly lignified biomass but the overestimated results obtained with woody biomass and winter-harvested grass indicate that further investigation of the hydrolysis of well-lignified biomass is required to improve the model precision. The model can easily be applied to energy-intense lipid- or protein-rich biomass with high precision.

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Figure Captions

- Figure 1. Specific CH\textsubscript{4} rate and VFA concentration plotted versus time course for 4 AD reactors. The average TAN for HRT of 15, 20, 30 and 45 days are 4.02, 3.88, 3.92 and 3.77 g kg ww\textsuperscript{-1}

- Figure 2. Degradation of crude lipids, crude proteins, hemicelluloses and cellulose. S\textsubscript{t}: content of organic compound residues; S\textsubscript{0}: content of initial organic compound

- Figure 3. Remaining fraction of hemicellulose and cellulose in prediction versus measured one (Above) and its simulation results of 200 days extended retention time.

- Figure 4. Model validation of the suggested BMP predicting model: Predicted versus measured BMP. The solid line indicates the best linear relationship (1:1). R\textsuperscript{2} of grass and crop residue including winter harvested sample 0.62; R\textsuperscript{2} of grass and crop residue excluding winter harvested samples 0.47; R\textsuperscript{2} of woody biomass 0.33; R\textsuperscript{2} of maize 0.00.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Table 1. Concentration of major organic compounds, methane potentials measured (BMP), stoichiometry methane potential (TBMP), pig manure, processed meat waste, brewery wastewater, slaughterhouse waste and feed mixture.

<table>
<thead>
<tr>
<th></th>
<th>Pig manure</th>
<th>Processed meat waste</th>
<th>Brewery wastewater</th>
<th>Slaughterhouse waste</th>
<th>Feed mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (g kg ww⁻¹)</td>
<td>39.55 (±0.96)</td>
<td>278.71 (±3.76)</td>
<td>7.74 (±0.97)</td>
<td>324.29 (±4.88)</td>
<td>70.0</td>
</tr>
<tr>
<td>VS (g kg ww⁻¹)</td>
<td>30.41 (±0.45)</td>
<td>239.08 (±4.57)</td>
<td>6.23 (±0.10)</td>
<td>314.20 (±5.02)</td>
<td>58.26</td>
</tr>
<tr>
<td>TKN (g kg ww⁻¹)</td>
<td>3.14 (±0.10)</td>
<td>19.09 (±0.61)</td>
<td>0.09 (±0.00)</td>
<td>7.66 (±0.41)</td>
<td>4.70</td>
</tr>
<tr>
<td>TAN (g kg ww⁻¹)</td>
<td>2.15 (±0.17)</td>
<td>0.00 (±0.00)</td>
<td>0.08 (±0.00)</td>
<td>1.66 (±0.12)</td>
<td>1.66</td>
</tr>
<tr>
<td>VFAs (g kg ww⁻¹)</td>
<td>7.12 (±0.85)</td>
<td>0.00 (±0.00)</td>
<td>0.29 (±0.09)</td>
<td>2.53 (±0.11)</td>
<td>5.44</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>23.41 (±2.43)</td>
<td>0.00 (±0.00)</td>
<td>0.81 (±0.15)</td>
<td>4.65 (±0.79)</td>
<td>9.33</td>
</tr>
<tr>
<td>Ethanol (g kg ww⁻¹)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>3.74 (±0.36)</td>
<td>0.00 (±0.00)</td>
<td>0.42</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>60.03 (±1.75)</td>
<td>0.00 (±0.00)</td>
<td>0.72</td>
</tr>
<tr>
<td>XP (g kg ww⁻¹)</td>
<td>6.19 (±0.46)</td>
<td>120.00 (±2.35)</td>
<td>0.00 (±0.00)</td>
<td>37.45 (±1.13)</td>
<td>19.08</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>20.35 (±0.55)</td>
<td>50.19 (±1.82)</td>
<td>0.00 (±0.00)</td>
<td>11.92 (±0.66)</td>
<td>32.74</td>
</tr>
<tr>
<td>XL (g kg ww⁻¹)</td>
<td>0.00 (±0.00)</td>
<td>100.00 (±2.15)</td>
<td>0.00 (±0.00)</td>
<td>230.00 (±4.34)</td>
<td>17.00</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>0.00 (±0.00)</td>
<td>41.83 (±1.05)</td>
<td>0.00 (±0.00)</td>
<td>73.20 (±2.92)</td>
<td>29.18</td>
</tr>
<tr>
<td>Hemicellulose (g kg ww⁻¹)</td>
<td>4.72 (±0.54)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>3.54</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>15.52 (±0.95)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>6.08</td>
</tr>
<tr>
<td>Cellulose (g kg ww⁻¹)</td>
<td>7.32 (±0.46)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>5.49</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>24.07 (±1.96)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>9.42</td>
</tr>
<tr>
<td>Lignin (g kg ww⁻¹)</td>
<td>5.03 (±0.78)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>3.77</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>16.54 (±1.10)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>0.00 (±0.00)</td>
<td>6.48</td>
</tr>
<tr>
<td>Carbohydrate (g kg ww⁻¹)</td>
<td>0.03</td>
<td>19.08</td>
<td>2.20</td>
<td>44.22</td>
<td>3.52</td>
</tr>
<tr>
<td>(%) of VS</td>
<td>0.11</td>
<td>7.98</td>
<td>14.07</td>
<td>35.31</td>
<td>6.05</td>
</tr>
<tr>
<td>TBMP (NL CH₄ kg VS⁻¹)</td>
<td>473</td>
<td>706</td>
<td>602</td>
<td>862</td>
<td>635</td>
</tr>
<tr>
<td>BMP (NL CH₄ kg VS⁻¹)</td>
<td>297±8</td>
<td>605±15</td>
<td>577±20</td>
<td>821±14</td>
<td>513</td>
</tr>
<tr>
<td>TBMP (NL CH₄ kg ww⁻¹)</td>
<td>14.39</td>
<td>168.74</td>
<td>3.75</td>
<td>270.97</td>
<td>36.97</td>
</tr>
<tr>
<td>SMP (NL CH₄ kg ww⁻¹)</td>
<td>9.03 (±0.26)</td>
<td>144.70 (±5.35)</td>
<td>3.60 (±0.35)</td>
<td>258.03 (±5.34)</td>
<td>29.91</td>
</tr>
<tr>
<td>BMP/TBMP (%)</td>
<td>62.8</td>
<td>85.8</td>
<td>95.9</td>
<td>95.2</td>
<td>80.9</td>
</tr>
</tbody>
</table>

Figures in parentheses are standard deviations.

XP: Crude protein; XL: crude lipid;
SMP: Specific methane potential in terms of wet weight (NL CH₄ kg ww⁻¹)
NL: normal liter at 273 K, 1.013 bar
Table 2. The concentration of each compound is indicated as TBMP in terms of wet weight (NL CH₄ kg ww⁻¹)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>VFA</th>
<th>Ethanol</th>
<th>XP</th>
<th>XL</th>
<th>Hemicellulose</th>
<th>Cellulose</th>
<th>Lignin</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig manure</td>
<td>2.66</td>
<td>0.00</td>
<td>3.07</td>
<td>0.00</td>
<td>1.96</td>
<td>3.04</td>
<td>3.66</td>
<td>0.01</td>
</tr>
<tr>
<td>Processed meat</td>
<td>0.00</td>
<td>0.00</td>
<td>59.47</td>
<td>101.36</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>7.91</td>
</tr>
<tr>
<td>Brewery wastewater</td>
<td>0.11</td>
<td>2.73</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.91</td>
</tr>
<tr>
<td>Slaughterhouse waste</td>
<td>0.94</td>
<td>0.00</td>
<td>18.56</td>
<td>233.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>18.34</td>
</tr>
<tr>
<td>Feed for reactor</td>
<td>2.03</td>
<td>0.31</td>
<td>9.45</td>
<td>17.23</td>
<td>1.47</td>
<td>2.28</td>
<td>2.74</td>
<td>1.46</td>
</tr>
</tbody>
</table>
Table 3. Hydrolysis rate, ultimate biodegradable and inert fraction of hemicellulose, cellulose and lignocellulose from the first-order kinetic regression test and its model calibration results

<table>
<thead>
<tr>
<th></th>
<th>$k_i$</th>
<th>$df_\infty$</th>
<th>(100-$df_\infty$)</th>
<th>$RMSE_C$</th>
<th>$rRMSE_C$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemicellulose(VS$_{HE}$)</td>
<td>0.0298</td>
<td>59.3</td>
<td>40.7</td>
<td>1.46</td>
<td>4.60</td>
<td>0.97</td>
</tr>
<tr>
<td>Cellulose (VS$_{CE}$)</td>
<td>0.0699</td>
<td>36.3</td>
<td>63.7</td>
<td>0.48</td>
<td>1.65</td>
<td>0.99</td>
</tr>
<tr>
<td>Lignocellulose</td>
<td>0.0602</td>
<td>27.4</td>
<td>72.6</td>
<td>0.77</td>
<td>3.68</td>
<td>0.99</td>
</tr>
</tbody>
</table>

$df_\infty$ is the ultimate biodegradable fraction (%); (100-$df_\infty$): inert fraction (%); $k_i$: the decay rate constant for a fraction (day$^{-1}$); $\theta$: HRT (days).

$RMSE_C$: Root Mean Square error in model calibration (NL CH$_4$ kg VS$^{-1}$)

$rRMSE_C$: Relative Root Mean Square error in model calibration (%)


Table 4. Model validation results of suggested BMP predicting model using the 4 datasets of samples used in this study.

<table>
<thead>
<tr>
<th>Tested substrates</th>
<th>$BMP_m$ (NL CH$_4$ kg VS$^{-1}$)</th>
<th>$BMP_p$ (NL CH$_4$ kg VS$^{-1}$)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig manure</td>
<td>305</td>
<td>297</td>
<td>5.5</td>
</tr>
<tr>
<td>Processed meat</td>
<td>639</td>
<td>605</td>
<td>24.0</td>
</tr>
<tr>
<td>Slaughterhouse waste</td>
<td>847</td>
<td>821</td>
<td>18.3</td>
</tr>
<tr>
<td>Brewery wastewater</td>
<td>602</td>
<td>577</td>
<td>17.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$RMSE_p$ (NL CH$_4$ kg VS$^{-1}$)</td>
<td>25.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$rRMSE_p$ (%)</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$BMP_m$: measured BMP (NL CH$_4$ kg VS$^{-1}$)  
$BMP_p$: predicted BMP (NL CH$_4$ kg VS$^{-1}$)  
$RMSE_p$: Root Mean Square error in prediction (NL CH$_4$ kg VS$^{-1}$)  
$rRMSE_p$: Relative Root Mean Square error in prediction (%)  
SD: Standard deviation between measured and predicted BMP
Table 5. Model errors in prediction using 64 external dataset

<table>
<thead>
<tr>
<th>Biomass type</th>
<th>Oslaj et al. (2010)</th>
<th>Triolo et al. (2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(^a)</td>
<td>Maize</td>
</tr>
<tr>
<td>(BMP_m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean) (NL CH(_4) kg VS(^{-1}))</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>(min) (NL CH(_4) kg VS(^{-1}))</td>
<td>251.4</td>
<td>104.0</td>
</tr>
<tr>
<td>(max) (NL CH(_4) kg VS(^{-1}))</td>
<td>294.7</td>
<td>391.3</td>
</tr>
<tr>
<td>SD(^b)</td>
<td>26.0</td>
<td>77.3</td>
</tr>
</tbody>
</table>

Validation results

| \(RMSE_p\) (NL CH\(_4\) kg VS\(^{-1}\)) | 37.4          | 49.9          | 43.7              | 86.1  |
| \(rRMSE_p\) (%)                          | 12.1          | 19.6          | 15.8              | 47.8  |

\(^a\) n : number of dataset used for validation.

\(^b\) SD: Standard deviation of BMP of attended dataset for validation (n).
Highlights

- We explored a new approach to determine biochemical methane potentials.
- The model was validated using 65 internal and external datasets.
- 45% of hemicelluloses and 34% of cellulose decomposed despite long HRT.
- The relative error for precision of the best model was 12.1%.
- The model has better performance for gently lignified biomasses.