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1 Characterization and prediction of organic nitrogen

biodegradability during anaerobic digestion: a bioaccessibility approach.

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13 Abstract

4

14 Prediction of organic nitrogen mineralization into ammonium during anaerobic digestion is required for optimizing substitution of mineral fertilizer by digestates. The aim of this study was to understand 15 organic nitrogen biodegradability and to investigate how it can be predicted from carbon 16 biodegradability, and nitrogen bioaccessibility, respectively. Bioaccessibility was assessed using 17 18 fractionation methods based on sequential extractions. Results showed that organic nitrogen was present in fractions whose bioaccessibility levels differed. Organic nitrogen and carbon 19 biodegradability were also determined and compared. Results highlighted two groups of substrates: 20 the first with an initial $NH_4^+/TKN < 30\%$, whose carbon and nitrogen biodegradability are similar; the 21 22 second with an initial $NH_4^+/TKN > 30\%$, whose carbon and nitrogen biodegradability differ 23 significantly. To enable prediction on all substrates, partial least square (PLS) regressions were carried 24 out to link organic nitrogen bioaccessibility indicators to biodegradability. The models successfully 25 predicted organic nitrogen biodegradability with a maximum prediction error of 10%. 26

27 Keywords : anaerobic digestion, nitrogen, ammonium, biodegradability, bioaccessibility

28 1. INTRODUCTION

Anaerobic digestion is currently attracting considerable interest for the management of agricultural 29 30 waste. The main objectives are reducing greenhouse gas emissions, procuring additional income for farmers and autonomy regarding nutrients. But the expansion of anaerobic digestion plants creates 31 32 new challenges concerning the management of the digestate. Indeed, residues from anaerobic digestion are rich in nutrients, particularly in nitrogen and are therefore of agronomic interest for their 33 34 value as fertilizer and amendment (Alburquerque et al., 2012; Tambone et al., 2009). Digestates from agricultural plants are mostly spread on fields after storage or post-processing (phase separation, 35 composting, etc.) with the aim of replacing mineral fertilizers. Indeed, anaerobic digestion results in 36 the mineralization of part of the organic nitrogen into ammonium (Möller and Müller, 2012), enabling 37 38 short term uptake of nitrogen by crops combined with long term mineralization of slowly biodegradable organic compounds (Fouda et al., 2013) in the soil. However, the digestate management 39 chain is subject to nitrogen leakages in the form of ammonia emissions during storage (Sommer, 1997) 40 and after spreading on the land, or through nitrate leaching (Möller, 2015). In addition to their 41 42 environmental impacts, nitrogen losses after digestion reduce the fertilizing value of the digestates and negatively impact the goal of replacing mineral fertilizers. Hence, to mitigate environmental impacts 43 and to optimize nitrogen uptake by crops, the anaerobic digestion process has to be designed taking 44 45 the characteristics of the digestate into account, i.e. ammonia content and organic nitrogen content, 46 together with short or long-term mineralization kinetics in soils. To this end, a detailed understanding of the nitrogen composition of digestates and anaerobic biodegradability of the organic nitrogen in the 47 substrates is required. 48

Nitrogen is present in substrates in two main forms: mineralized (e.g. ammonium, nitrates, etc.) and organic. Anaerobic digestion results in the degradation of part of the organic nitrogen into ammonium, a reduced form (González-Fernández and García-Encina, 2009). In the substrates, the organic nitrogen is mainly contained in proteins and requires hydrolysis to be converted into monomers. In most cases, the amino acids that result from this transformation are then metabolized through acidogenesis to produce volatile fatty acids and ammonium via Stickland or single amino acid fermentation (Ramsay and Pullammanappallil, 2001). However, some authors studied organic nitrogen biodegradation and

56 showed that degradation of the organic nitrogen was not complete during anaerobic digestion, but rather ranged from 18% to 77% depending on the substrate and the hydraulic retention time (HRT), as 57 58 reported in the different studies (Kobayashi et al., 2012, 2014; Massé et al., 2007; Yang et al., 2015). These results highlight the fact that protein biodegradation is strongly constrained by the 59 bioaccessibility of organic nitrogen. 60 Accessible organic matter refers to the compounds in the substrate that microorganisms can access for 61 62 intra or extra-cellular degradation. A number of bioaccessibility indicators have been developed using chemical extraction or particle size distribution (Dulekgurgen et al., 2006; Karahan et al., 2008) to 63 fractionate organic matter according to its degree of bioaccessibility. Better characterization of the 64 bioaccessibility of organic nitrogen in substrates will enable a better understanding of the variability of 65 66 its anaerobic biodegradability. Methods using successive chemical extractions such as the Van Soest method, or more advanced methods can be used for this purpose. 67 The Van Soest fractionation method (Van Soest, 1963) is widely used to fractionate wastes including 68 vegetables, sludges, and plants. It uses different solvents to extract soluble and lignocellulosic 69 70 compounds. The acidity of the solvents is increased at each step by using hot water, neutral detergent, acid detergent and finally strong acid extraction. The different extraction steps target soluble, protein-71 like, hemicellulose-like and cellulose-like compounds, respectively. The residual organic matter is 72 73 considered as lignin-like compounds. Many studies have explored the correlations between the Van 74 Soest composition of organic matter and its biodegradability (Gunaseelan, 2007; Liu et al., 2015; Mottet et al., 2010). Gunaseelan (2007) demonstrated an inverse relationship between the 75 lignin+cellulose-like content and biodegradability of fruit and vegetables in anaerobic conditions. 76 77 Despite these promising results, Mottet et al., (2010) pointed out that this fractionation method is too 78 aggressive to be representative of biological disintegration that occurs during anaerobic digestion, 79 which limits its use for the prediction of biodegradability. Jimenez et al., (2015) then developed a less 80 aggressive fractionation method based on the protocol of Muller et al., (2014) that targeted extracellular polymeric substances. This protocol, named EPS protocol, uses increasing alkaline 81 solutions, and targets protein-like substances; it ends with strong acid extraction targeting 82

83 holocellulosic-like products. This fractionation, combined with 3D-fluorescence to assess the

84 complexity of each fraction, successfully predicted the bio-methanogenic potential (BMP) and carbon biodegradability of various substrates using partial least square (PLS) regressions (Jimenez et al., 85 86 2017). Hence, EPS fractionation combined with PLS regression enables the fast determination of the BMP value and carbon biodegradability by decreasing the time for analysis from 40-50 days (for a 87 BMP) to 3 days (for EPS fractionation). However, all these studies focused on the biodegradability of 88 volatile solids or carbon compounds. Consequently, no data are available concerning the ability of 89 90 these accessibility indicators, determined from EPS or Van Soest fractionation, to predict organic 91 nitrogen biodegradability during anaerobic digestion.

92 The aim of this study was thus (i) to investigate the biodegradability of organic nitrogen in mesophilic anaerobic codigestion and (ii) to develop predictive models using carbon biodegradability 93 94 and organic nitrogen bioaccessibility as variables, respectively. To this end, we first investigated the bioaccessibility of organic nitrogen on various substrates and compared it with carbon bioaccessibility 95 using two fractionation methods. Next we compared the anaerobic biodegradability of nitrogen and 96 carbon on the same panel of substrates. Finally, we performed PLS regressions to identify possible 97 98 correlations between organic nitrogen biodegradability and bioaccessibility indicators provided by the two fractionation methods to determine which fractionation method better describes organic nitrogen 99 biodegradability. 100

101

102 2. MATERIAL AND METHODS

103 **2.1. Substrates**

Twenty different substrates were collected in France as representative of the diversity of organic
wastes used in agricultural anaerobic digestion plants. They included livestock wastes, wastes from
agri-industry and municipalities and agriculture residues. Table 1 lists the substrates sampled and their
characteristics. Substrate panel was selected to cover a large range of protein content and C/N ratio.
Hence, protein content in this panel ranges from 12 to 67% org-C and C/N ratio from 5.7 to 36.
All the samples were ground in a knife mill (R15 V.V., Robotcoupe®) and stored at -20 °C. Samples
for elemental analysis were ground in a ball mill (Dangoumill®, PROLABO®).

112	2.2. Physico-chemical characterization
113	Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN) and total ammonia nitrogen were
114	measured using standard methods (APHA, 2005). TKN and ammonia concentrations were determined
115	by distillation (TKN after mineralization). Total carbon and total nitrogen were measured in finely
116	ground raw samples by elemental analysis according to the manufacturer's instructions (Thermoflash
117	2000, Thermoscientific®). Inorganic carbon in the raw samples was determined by calcination at 480
118	°C and elemental analysis of the ashes. Total organic carbon (TOC) was determined by subtracting
119	inorganic carbon (IC) from total carbon (TC). Protein content in organic carbon was calculated using
120	the following the equation (1):
121	(1) Protein = $((TKN-NH_4^+)*C/N_{protein})/TOC$
122	Where:
123	- Protein: protein content (% _{org-C}).
124	- $C/N_{protein}$: carbon/nitrogen ratio equal to 4.348 gC.gN ⁻¹ based on Ramsay and
125	Pullammanappallil (2001).
126	
127	2.3. Organic matter fractionation
128	The fractionation methods used in this study to characterize organic matter were based on sequential
129	chemical extractions. The successive steps of the two fractionation methods, derived from EPS
130	(Jimenez et al., 2015) and Van Soest (Van Soest, 1963), are described below. Four fractions were
131	extracted with each method and presented similarities in terms of molecules targeted by the extracting
132	solvents as presented in table 2, which made it possible to compare the two methods. Uncertainty on
133	fractionation results was estimated for each extraction method using a Monte Carlo method in order to
134	take error propagation into account.
135	
136	2.3.1. Modified Van Soest fractionation
137	The modified Van Soest fractionation is based on successive extractions with neutral and acid
138	detergents and ending with a strong acid extraction. Organic matter was separated into four fractions:

139	• SOL_VS: This fraction is extracted in water at a temperature of 20 °C for 1 h. The extraction
140	temperature is different from that described in the extraction protocol for the French standard
141	XPU 44-162 (AFNOR) where extraction is performed at 100 °C.
142	• PROT_VS : This fraction is extracted with boiling commercial neutral detergent (NDF: 1.86%
143	disodium ethylene diamine tetraacetate, 3% aqueous solution of sodium dodecyl sulfate,
144	0.68% decahydrate sodium tetraborate, 0.45% disodium hydrogen phosphate and 1.0%
145	triethylene glycol; Carlo Erba®) for 1 h from the pellet obtained after the SOL_VS step.
146	• HOLO_VS: This fraction results from the two successive extractions of the Van Soest
147	protocol: (1) extraction with boiling commercial acid detergent ADF (2.6% sulfuric acid and
148	2.0% cetrimonium bromide; Carlo Erba®) for 1 h, followed by (2) extraction with 72%
149	sulfuric acid (w/w) at ambient temperature for 3 h. This step used the pellet remaining from
150	the PROT_VS step.
151	• LIGN_VS: This fraction contained the remaining materials not extracted during the previous
152	extractions.
153	After the first extraction, the mix was centrifuged at 12100 g for 20 minutes. The supernatant
154	containing the soluble fraction of organic matter was removed and the remaining pellet was then dried
155	at 40°C and then ground to 1 mm. Detergent and strong acid extraction (PROT_VS and HOLO_VS
156	steps) were performed on 1 gram of the dried-ground sample sealed in filter bags (F57 Filter Bags,
157	Ankom Technologies®). Detergent and acid extractions were performed using an automated fiber
158	analyzer (Ankom 2000, Ankom Technologies®). After each extraction step, the content of one bag
159	was used to analyze total organic carbon and total remaining nitrogen using an elemental analyzer
160	(Thermoflash 2000, Thermoscientific®). The organic carbon and nitrogen content in each fraction
161	were determined by the difference between the organic carbon and nitrogen content in the filter bags
162	before and after each extraction.
163	

163

164 2.3.2. Modified "EPS" fractionation

EPS fractionation (Jimenez et al., 2015) is based on successive alkaline extractions ending with strongacid extraction. This fractionation method is similar to the Van Soest method including the use of

strong acid extraction at the end. Sequential extractions were performed on raw samples (0.5 gram of
dry matter) using the method described by Jimenez et al. (2017). This method separates the organic
matter into four fractions:

SOL_EPS: The first step of the protocol consists of two successive extractions of the sample
with 30 mL of a saline solution of CaCl₂ 10mM at 30 °C under stirring (300 rpm) for one
hour.

- PROT_EPS: The pellet remaining from the first step is washed four times with 30 mL of a
 basic solution of NaOH 10mM + NaCl 10mM at 30 °C and under stirring (300 rpm) for 15
 minutes. The remaining pellet is washed four times with 30 mL of a strong basic solution of
 NaOH 0.1M at 30 °C and under stirring (300 rpm), for one hour.
- HOLO_EPS: The pellet remaining from the previous step is washed three times with 25 ml of
 a strong acid solution of H₂SO₄ 72% (w/w) at 30 °C under stirring at 300 rpm for three hours.
 This step is equivalent to the final extractions carried out in Van Soest protocol used to extract
 holocellulose materials,
- LIGN_EPS: This fraction contains the most recalcitrant material and is calculated by
 subtracting the total mass extracted from the original mass of the sample.

183 The organic carbon and total nitrogen content in each fraction (except LIGN_EPS fraction) were 184 measured in the extracts using a TOC-L analyzer (Shimadzu®) and by carbon and nitrogen balance for 185 LIGN_EPS fraction between the raw sample and the sum of the extractible fractions. All extractions 186 and measurements on extracts were performed in triplicate.

187

188

2.4. Organic carbon and nitrogen biodegradability

Anaerobic biodegradability of organic carbon and nitrogen were analyzed on each substrate studied. The substrate was prepared in the same way as the one used before the extraction protocol (described in section 2.1). The inoculum used for both experiments was obtained from a continuous mesophilic anaerobic digester that is used to process pig slurry and horse feed. Carbon biodegradability was assessed in processing biochemical methanogenic potential (BMP) experiments according to Vedrenne et al., (2008). These batch biodegradability experiments were performed in triplicate with an

inoculum:substrate ratio of 1 gVS.gVS⁻¹ and were incubated at 38 °C for 40 days. TC and biogas
production were used to determine carbon mass balance continuity during the monitoring. Whatever
the test, mass balance error is between -8 and 10%.

ANMP (Apparent nitrogen mineralized potential) tests were carried out to determine the maximum 198 apparent organic nitrogen mineralized during anaerobic digestion. This method allows the 199 determination of the organic nitrogen part of the substrate converted into soluble ammonium which is 200 201 the result of organic nitrogen mineralization from substrate, biomass growth and biomass decay. The experimental conditions were similar to the BMP experiments and the ammonium concentration was 202 monitored during the test. However, the inoculum used for ANMP tests was previously centrifuged at 203 12100 g for 20 minutes at ambient temperature and the supernatant was removed in order to reduce the 204 205 initial ammonium concentration in the batch reactors. The remaining pellet was suspended in a KHCO₃ 10 g.L⁻¹ + NaHCO₃ 10 g.L⁻¹ solution to preserve sufficient buffering capacity in the batch and 206 to prevent inhibitions due to acidification during incubation. A blank experiment was performed 207 without the addition of substrate to assess the apparent organic nitrogen mineralization due to the 208 209 inoculum. The AMNP of each substrate was determined by difference between apparent mineralization in the batch experiment with a substrate and the control experiment, as described by 210 equation (2). 211

212 (2) $AMNP = \frac{NH_{4-substrate}^{+} - NH_{4-blanck}^{+} - NH_{4-lnitial substrate}^{+}}{TS_{substrate}} - NH_{4-lnitial substrate}^{+}$

213 Where:

214 AMNP : Apparent Nitrogen Mineralized Potential of the substrate $(gN-NH_4^+.kgTS_{substrate}^{-1})$ 215 $NH_{4-substrate}^+$: total mass of ammonium into the batch experiment with substrate after 216 incubation $(gN-NH_4^+)$

- 217 $NH_{4-blank}^+$: total mass of ammonium into the blank batch experiment without substrate after 218 incubation (gN-NH₄⁺)
- 219 *MV_{substrate-added}*: total mass of the volatile matter of the substrate added to the batch
 220 experiment (kgTS_{substrate})

221

NH⁺_{4-Initial substrate} : Ammonium concentration in the substrate before incubation (gN-222 NH_4^+ .kgTS_{substrate}⁻¹).

Ammonium and TKN contents were measured before and after each incubation test using the 223 distillation method to check nitrogen mass balance. The ammonium distillation method is aggressive 224 225 enough to solubilize ammonium precipitates such as struvite and these ammonium forms are included in total ammonium. Hence, ANMP value considers organic nitrogen mineralization into ammonium 226 227 which can be soluble or precipitated in the digestate. Whatever the test, difference between initial and final total nitrogen content is between -4% and 5%. 228

ANMP is an apparent mineralization potential which considers organic nitrogen from substrate 229

mineralization and ammonium immobilization for biomass growth. To quantify the impact of 230

ammonium immobilization for biomass growth on ANMP value, a control batch experiment was 231

232 performed following the ANMP protocol with a volatile fatty acid solution as substrate. This

experiment highlights an immobilization rate of 0.006 gN-NH₄⁺_{immobilized}.gC_{degraded}⁻¹. This value is lower 233

than the references found in the literature that range from 0.017 to 0.009 gN-NH₄⁺ immobilized.gC_{degraded}⁻¹ 234

(Batstone et al., 2002; Ramsay and Pullammanappallil, 2001). However, these constants only consider 235

growth, while in this study, the constant is the result of both growth and partial decay during 40 days. 236

This constant was applied to the panel of substrate. Hence, $6 \pm 3\%$ of the TKN brought by the substrate 237

can be immobilized for biomass growth after 40 days of incubation. 238

239 All the biodegradability tests were performed in triplicate to determine uncertainties due to the 240 operator and the measuring equipment.

241

2.5. Statistical analysis 242

In order to sort substrates according to their biodegradation rate and investigate the relationship 243 between organic nitrogen biodegradability and organic nitrogen bioaccessibility, hierarchical cluster 244 analysis (HCA) and partial least square (PLS) regressions were performed on the data set using 245 Xlstat® for Excel®. Substrates were sorted depending on their organic carbon and organic nitrogen 246 biodegradability by using HCA. 247

Models based on fractionation methods were developed to describe and predict organic nitrogen 248 biodegradability using PLS regression. PLS regression is an extended multiple linear regression that 249 include principal component analysis principles in the regression structure. This technique build model 250 to link one dependent variable to different explanatory variables by minimizing their covariance. Here, 251 the dependent variable (data to predict) was organic nitrogen biodegradability ($gN-NH_4^+$, $kgTS^{-1}$) and 252 253 the explanatory variables (predictor variables) were the organic nitrogen content in each fraction for, in one case modified Van Soest fractionation (expressed in gN.kgTS⁻¹), and in another case, modified 254 EPS fractionation (expressed in gN.kgTS⁻¹). In this study, model robustness was investigated using the 255 following quality parameters: 256 The coefficient of determination R^2 , which is the proportion of the dependent variable 257 variance that can be explained by the explanatory variable set and varies between 0 and 1. 258 Based on previous studies using PLS for biodegradability prediction (Jimenez et al., 2017), a 259 coefficient of determination superior to 0.8 is required to ensure a good fitting of the 260 experimental data. 261 The cross validated coefficient of determination Q², which is the variation rate of the 262 dependent variable predicted by the model by cross validation. O² makes it possible to judge 263 264 the quality of the fit between the predicted and measured output variables and the ability of the model to predict new data. This coefficient varies between $-\infty$ and 1. A O² value above 0.5 265 indicates good predictability of the model (Jimenez et al., 2014). Cross-validation was done 266 using the jackknife method by excluding 1 out of 4 values in the data set chosen at random. 267 External model validation was done by applying the models to another set of data covering a wide 268 269 range of biodegradability and substrate origin. The predicted organic nitrogen biodegradability was 270 compared with the measured values.

271

3. RESULTS AND DISCUSSION

272 **3.1.** Organic carbon and nitrogen distribution in bioaccessibility based fractionation.

Modified Van Soest and modified EPS fractionation were compared on 20 substrates representing the
diversity of feedstock in agricultural anaerobic digestion plants. Figure 1 shows the results of Van
Soest and EPS fractionations for carbon content (1a) and organic nitrogen content (1b).

276

277 *3.1.1.* Fractionation using the modified Van Soest method

Figure 1a shows that the distribution of organic carbon in modified Van Soest fractions differed 278 considerably from one substrate to another, with SOL_VS ranging from 13%_{TOC} to 48%_{TOC}, 279 PROT_VS from 5%_{TOC} to 37%_{TOC} and HOLO_VS from 9%_{TOC} to 66%_{TOC}. Only LIGN_VS showed 280 281 limited distribution, ranging from $1\%_{TOC}$ to $11\%_{TOC}$. The wide range of values shows that the carbon distribution obtained with this fractionation method is strongly substrate dependent. In figure 1b, 282 organic nitrogen distribution shows that nitrogen was present in all fractions and, like carbon, is 283 strongly substrate dependent. Indeed, the ranges of nitrogen contents were wide in each fraction. On 284 285 average, only about 30% org-N was contained in the NPROT_VS fraction. The remaining organic nitrogen was mainly spilt between the NSOL_VS (35% org-N on average) and NHOLO_VS fractions 286 (30%_{org-N} on average). The range of nitrogen contents was wide in all fractions except NLIGN_VS. 287 Only a small proportion of organic nitrogen (between 1 and 15%_{org-N}) was contained in LIGN_VS. 288 289 Other authors have reported the presence of nitrogen in all the fractions, even in those that are hard to extract like HOLO VS and LIGN VS, using the Van Soest method for organic matter characterization 290 before composting (Denes et al., 2015) or soil mineralization of organic waste (Chalhoub et al., 2013; 291 292 Garnier et al., 2003).

293

3.1.2. Fractionation using the modified EPS method

Figures 1a and 1b show that variability of the carbon and nitrogen content of all EPS fractions was
also wide. Extractible fractions SOL_EPS, PROT_EPS and HOLO_EPS ranged from 2 to 31%_{TOC}, 3
to 20%_{TOC} and 10 to 45%_{TOC}, respectively, for organic carbon. Non-extractible LIGN_EPS ranged

from 29 to $59\%_{TOC}$. The distribution of organic nitrogen ranged from 2 to $36\%_{org.N}$ for NSOL_EPS, 3

299 to $41\%_{org-N}$ for NPROT_EPS, 3 to $29\%_{org-N}$ for NHOLO_EPS and from 17 to $67\%_{org-N}$ for 300 NLIGN_EPS. The wide range of variability obtained shows that the carbon distribution in the EPS 301 fractions is strongly substrate dependent. In addition, organic carbon and nitrogen are largely present 302 in the non-extractible fraction LIGN EPS and NLIGN EPS reflecting that the extraction method uses 303 soft extractants to target organic compounds. These soft extractants are supposed to solubilize specific molecules in organic matter, but organic nitrogen, which is mainly present in protein, was also 304 305 detected in HOLO_EPS and a large proportion in the LIGN_EPS fraction. According to Jimenez et al. (2015), HOLO_EPS and LIGN_EPS are mainly composed of lignocellulosic materials and other 306 307 materials entrapped by them. Lignocellulosic materials are hardly biodegradable and biodegradable molecules which are entrapped by them are hardly biodegradable as well (Monlau et al., 2012). Thus, 308 309 the organic nitrogen contained in HOLO_EPS and LIGN_EPS fractions is the result of the organic nitrogen entrapment by lignocellulosic materials that are not disintegrated by alkali solvents. 310 Consequently, this fractionation method not only takes into account the nature of the extracted 311 molecules, but also their bioaccessibility due to physical structure. 312 313 Comparison of carbon and nitrogen distribution obtained with the modified Van Soest 314 3.1.3. and modified EPS fractionation methods 315 316 The distributions of organic carbon and nitrogen obtained with modified EPS and modified Van Soest 317 fractionation methods differed significantly. Indeed, the percentage of organic carbon contained in extractible fractions was higher using modified Van Soest fractionation than the modified EPS 318 fractionation: HOLO_VS was 36%_{TOC} higher than HOLO_EPS and PROT_VS was 66%_{TOC} higher 319

320 than PROT_EPS. In contrast, the non-accessible fraction was noticeably higher with the modified EPS

321 method than with the modified Van Soest method. The LIGN_EPS fraction reached $41\%_{TOC}$ on

322 average while LIGN_VS represented only 6% TOC. The same kinds of results were obtained for organic

323 nitrogen fractionation even if the trend of distribution between extractible fractions differed

324 significantly. These observations show that a significant part of the organic carbon and nitrogen

325 extracted with the Van Soest method solvents was not extracted by EPS solvents but remained in the

326 non-extractible fraction LIGN_EPS.

327 To explain these results, the composition of solvents used for each fractionation method has to be considered. In particular, as stated by Jimenez et al. (2015) and Muller et al. (2014), EPS fractionation 328 329 aims to describe the bioaccessibility of organic matter considering the impact of its chemical composition and physical structure. However, Van Soest fractionation only considers the impact of 330 chemical composition on bioaccessibility (the ability of exoenzymes to reach active sites on organic 331 compounds considering its chemical complexity but with no limitation caused by the physical 332 333 structure of the organic matter). Indeed, as observed by Jimenez et al. (2015), Van Soest solvents are more aggressive than EPS solvents. In particular, the NDF solvent used for the second step of the Van 334 Soest fractionation is a detergent and results in strong disintegration of complex structures composed 335 by lignin and holocellulose. Thus, this extraction step allows the extraction of physically inaccessible 336 337 organic matter at this and subsequent steps. As a consequence, few carbon and nitrogenous compounds were present in LIGN_VS. On the other hand, the alkaline solvents used for EPS 338 fractionation did not disintegrate the organic matter and extracted only the physically and chemically 339 accessible compounds. These results indicate that, on average, 38% org-N of the organic nitrogen and 340 341 35% TOC of the organic carbon (estimated by difference between non-extractible fractions obtained for EPS and Van Soest fractionation respectively) are contained inside complex physical structures 342 containing lignocellulose compounds which strongly limits its bioaccessibility. 343 344 345 3.2. Organic carbon and nitrogen biodegradability under anaerobic conditions 3.2.1. Influence of substrate origin on biodegradability 346 Organic nitrogen biodegradability and carbon biodegradability were determined using the AMNP test 347 and the BMP test. The results are presented in table 3. 348 349 Organic nitrogen biodegradability is strongly substrate dependent, ranging from 14% org-N of 350 degradation to 82%_{org-N} in the substrate panel, with an average value of 48%_{org-N}. In comparison, 351 organic carbon biodegradability varied less, from 36 to $74\%_{TOC}$, with a mean value of 54%. 352 Biodegradability of organic nitrogen and carbon was shown to be partial, which is in accordance with the results previously obtained with the EPS fractionation method, which showed that a significant 353

354 part of the organic nitrogen and carbon is not bioaccessible. Indeed, the EPS method showed that, on average, 41% $_{\rm TOC}$ and 43% $_{\rm org\text{-}N}$ cannot be extracted. 355

To further investigate the variability of organic nitrogen and carbon biodegradability, substrate 356 categories can be defined using a statistical method. To this end, three indicators were used that 357 358 include the biodegradation rate for carbon and nitrogen, respectively, along with the NH₄⁺/TKN ratio 359 in the raw substrate. Hierarchical cluster analysis was performed on these variables for all substrates. 360 The dendrogram (fig. 2a) highlights two groups of substrates based on the variables.

361

3.2.2. Comparison between organic carbon biodegradability and organic nitrogen 362 biodegradability. 363

As highlighted in figure 3a, the first group G1 is mainly composed of substrates that present a slight 364 365 difference between organic carbon biodegradation and organic nitrogen biodegradation with high 366 biodegradability (57% on average for organic carbon and nitrogen). Figure 2b shows that G1 is also composed by substrates with a low NH_4^+/TKN ratio (< 30%) and includes all crop residues, non-stored 367 368 agro-industrial wastes and one silage sample out of three. As the biodegradability values for organic 369 carbon and nitrogen are very close for these kinds of substrates, which contain less than 30% of 370 mineralized organic nitrogen (fig.3a), predicting organic nitrogen biodegradation from organic carbon mineralization is possible with a mean absolute error of 12%. Indeed, some exceptions remain, like 371 supermarket vegetable wastes as well as drain screenings from slaughterhouses with a percentage 372 difference 39% and 37%, respectively between organic carbon and nitrogen biodegradability. 373 374 As shown in figures 2a, 2b and 3b, the second group, G2, is the opposite and is composed of substrates 375 with a marked difference in the biodegradability of organic carbon and nitrogen, and with a high 376 NH_4^+/TKN ratio (> 30%). Livestock manure is the most representative of this group but silage and 377 stored agro-industrial waste show the same behavior. The biodegradation levels of these substrates 378 containing large amounts of nitrogen in mineralized form are lower than for G1, with average carbon 379 and nitrogen biodegradability of 43% and 30%, respectively. In addition, the biodegradability of 380 organic carbon and nitrogen are not correlated in this type of substrate, making it impossible to 381 accurately predict organic nitrogen biodegradability from the value obtained for organic carbon.

382 The NH4+/TKN ratio indicates if organic nitrogen mineralization occurs before anaerobic digestion or not. As highlighted by the results, these transformations can occur during long storage (supermarket 383 384 vegetable waste), animal digestion (livestock manures) or silage fermentation process (crop silage samples). During these processes, carbon and nitrogen mineralization can differ. As an example, 385 during crop silaging, fermentation produces organic acids. These organic acids such as lactic acid lead 386 387 to a pH decrease and an inhibition of methanogenic activity (Palmisano and Barlaz, 1996). Hence, 388 transformations of organic carbon (partial hydrolysis and acidogenesis) occur but without significantly reaching mineralization and most of the BMP is preserved. However, as highlighted by Teixeira 389 Franco et al. (2016), the proteolytic activity can continue and a large amount of ammonia can be 390 produced indicating a significant organic nitrogen mineralization. This process can significantly 391 392 impact AMNP value. Hence, the impact of silage fermentation process on BMP and ANMP values can 393 significantly differ. Even if processes differ, animal digestion and spontaneous transformations during storage can have a differential impact on BMP and ANMP values. Therefore, the prediction of organic 394 nitrogen biodegradability from the value obtained for organic carbon (derived from BMP values) is 395 396 not relevant for most of the substrates belonging to the second group G2. Hence, depending on transformations that can occur before anaerobic digestion, a substrate can move from G1 (fresh 397 substrates) to G2. In the results, this phenomenon is observed for oat that belongs to G1 after harvest 398 399 and to G2 after silage fermentation process.

400 The links between substrate typology and substrate characteristics (protein content, C/N ratio, organic nitrogen fractionation following modified Van Soest and EPS methods) were investigated. C/N ratio, 401 402 protein content and organic nitrogen fractionation following modified Van Soest method are not 403 significantly different for G1 and G2 (data not shown). However, as presented in figure 4, significant 404 differences are observed on EPS fractionation obtained for G1 and G2. Indeed, carbon fractionation 405 results are close for both groups (fig.4a), but, organic nitrogen fractionation shows significant 406 differences between substrates belonging to G1 and G2, respectively (fig.4b). Hence, NSOL_EPS 407 fraction for G2 (7% or ... in average) is significantly inferior to the average value obtained for G1 (27%_{org-N}). In addition, the relative part of NLIGN_EPS for G2 (51%_{org-N} in average) is higher than 408 for G1 (39% org-N in average). Differences between G1 and G2 are less significant for NPROT_EPS and 409

410	NHOLO_EPS fractions. These results highlight that for substrates belonging to G2, transformations
411	prior to anaerobic digestion (silage fermentation, storage or animal digestion) led to the mineralization
412	of most of the bioaccessible organic nitrogen (NSOL_EPS fraction). Resulting substrates contain more
413	hardly bioaccessible organic nitrogen (NLIGN_EPS fraction). These changes in bioaccessibility of
414	organic nitrogen explain why average ANMP for G2 is significantly inferior to the average value
415	obtained for G1. In addition, results highlight that the impact of transformations taking place before
416	anaerobic digestion (silage fermentation, storage or animal digestion) impact differently organic
417	carbon and nitrogen bioaccessibility, which can explain why prediction of organic nitrogen
418	biodegradability from the value obtained for carbon is impossible for G2. These results highlight that
419	the modified EPS fractionation allowed the determination of accessibility indicators which can explain
420	biodegradability variations for organic nitrogen. Thus, using a fractionation method and especially
421	modified EPS fractionation could help predict organic nitrogen biodegradability.
422	
423	3.3. Prediction of organic nitrogen biodegradability from bioaccessibility indicators
424	3.3.1. Partial least squares (PLS) regression for the prediction of organic nitrogen
425	biodegradability
426	PLS analysis was first carried out on the whole set of substrates. Secondly, the same analysis was
427	performed on previously determined substrate groups (G1 end G2, see section 3.2), respectively.
428	
429	3.3.1.1. Development of the statistical model considering the whole substrate set
430	Two PLS analysis were carried out to investigate correlations between organic nitrogen
431	biodegradability and the bioaccessibility indicators obtained with the modified Van Soest and
432	modified EPS fractionations, respectively, considering the whole set of substrates. Two statistical
433	models were obtained (see Table 4): Model N_{EPS} , which considers modified EPS fractions of organic
434	nitrogen as explanatory variables, and model $N_{\text{VS}},$ which considers modified Van Soest ones as
435	explanatory variables. Both models show good model structure according to the cross validated
436	coefficient of determination Q^2 ($Q^2 > 0.5$) with $Q^2(N_{EPS}) = 0.736$ and $Q^2(N_{VS}) = 0.571$. However, the
437	proportion of the organic nitrogen biodegradability variance that can be explained by the

438 bioaccessibility indicators is higher with the N_{EPS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than with the N_{VS} model ($R^2 = 0.789$) than $R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) than $R^2 = 0.789$ model ($R^2 = 0.789$) the $R^2 =$

0.640). The N_{EPS} model using modified EPS fractionation thus enables better simulation of the

440 variation in nitrogen biodegradability, but R² is inferior of 0.8 so taking into account the typology

determined in section 3.2. could improve the predictability of the models.

- 442
- 3.3.1.2. Statistical models for the prediction of organic nitrogen biodegradability considering the
 set of substrates and associated quality parameters considering substrate group G1 and
 G2
- As mentionned in section 3.2., the substrates can be divided into two groups, G1 (ratio of $NH_4^+/NTK <$ 446 30%) and G2 (ratio of $NH_4^+/NTK > 30\%$) whose organic nitrogen mineralization patterns differ. PLS 447 448 analyses were thus performed to take them into account. Models considering modified EPS fractions 449 as explanatory variables are N_{EPS G1} for G1 and N_{EPS G2} for G2 and are detailed in Table 5. Quality parameters shows that the models considering the two typologies are better than for the model N_{EPS} 450 $(Q^2 = 0.736 \text{ and } R^2 = 0.789)$. Hence, model N_{EPS_G1} describing the biodegradability of the organic 451 452 nitrogen for G1 shows $Q^2 = 0.802$ and $R^2 = 0.843$. Model N_{EPS_G2} which considers substrates from G2 shows $Q^2 = 0.771$ and $R^2 = 0.943$. Considering models found in literature developed using PLS 453 regression, N_{EPS G1} and N_{EPS G2} models have satisfying R² values (0.818 for Jimenez et al. (2017), 0.88 454 for Monlau et al. (2012) and 0.925 for Wold et al. (2001)). These results reveal that substrate typology 455 456 enables better accuracy and predictability with models using EPS fractionation as explanatory variables. 457

Models considering modified Van Soest fractions as explanatory variables (N_{VS G1} for G1 and N_{VS G2} 458 459 for G2, see Table 5) present no real improvement of the R^2 with 0.668 and 0.455 for respectively 460 N_{VS G1} and N_{VS G2}. These results showed that the variance of organic nitrogen biodegradability is not 461 well described considering the typology of substrate by models using modified Van Soest variables. 462 Also, the cross-validation parameter Q² obtained for N_{VS G1} (0.551) and for N_{VS G2} (0.057) were below the value obtained for model N_{VS} (0.571) which took the whole set of substrates into account. These 463 results indicate that the substrate typology does not improve the robustness of statistical models 464 considering Van Soest variables. Consequently, prediction of organic nitrogen biodegradability with 465

models using the modified Van Soest fractionation as explanatory variables is not accurate. This result
is due to the lack of information about the physical structure of substrates even though this data
strongly impacts bioaccessibility. This statement is mainly due to the use of detergents during
extractions that lead to the complete disintegration of the physical structure of the organic matter as
stated by Mottet et al. (2010).

471

472 3.3.1.3. External model validation on models considering modified EPS variables For external model validation, the ability of each model developed to predict organic nitrogen 473 biodegradability of four substrates not used for model development was investigated. Predicted 474 organic nitrogen biodegradability and prediction errors were only determined for models that showed 475 476 satisfying robustness in the previous sections (N_{EPS}, N_{EPS_G1}, N_{EPS_G2}). Results are presented in Table 6. Regarding the prediction of organic nitrogen biodegradability by models using modified EPS fractions 477 as explanatory variables, the prediction errors of the N_{EPS} model that considers the whole set of 478 479 substrates, range from 5% to 37%. These results are better than those obtained with models using 480 modified Van Soest fractions as explanatory variables but nevertheless underline the poor predictibility of the N_{EPS} model. However, models that were specifically designed for each group of 481 substrates, G1 and G2, produced much better results. The N_{EPS_G1} and N_{EPS_G2} models that predict 482 483 organic nitrogen biodegradability for substrates belonging to the groupe G1 and G2 respectively, 484 produced very accurate predictions with a maximum prediction error of 2% and 10%, respectively. Hence, to accurately estimate organic nitrogen biodegradability, models have to consider modified 485 486 EPS fractions as explanatory variables and G1 and G2 as validity domains. 487 The EPS fractionation method has been used to develop accurate models for BMP prediction on 488 various substrates (Jimenez et al., 2017). These models were completed with complexity variables 489 acquired with 3D fluorescence spectrometry and reach a R^2 of 0.818 and a $Q^2 = 0.689$. The 490 performance of the models developed in this study were similar without considering complexity, 491 which can be explained by sampling before extraction and the type of substrate considered. Indeed, the preparation of the sample by Jimenez et al. (2017) (freeze-drying + grinding to 1 mm) modifies the 492 physical structure and complexity, but using 3D fluorescence spectrometry makes it possible to track 493

the recalcitrant materials released in the different fractions. The samples in the present study were used raw with only light pretreatment as described in section 2.3.1 which saved the structure of organic matter, and only bioaccessible materials were released during the different extraction steps. These results highlight the fact that EPS fractionation allows the determination of bioaccessibility indicators that take the impact of physical structure of the substrate organic matter into account in its biodegradability.

500

3.3.1.4. Analysis of the impact of bioaccessibility indicators on nitrogen biodegradability 501 The normalized coefficient of each variable of the model can be determined from the correlation 502 between the explanatory variable and the dependant variable, hence, based on these coefficients, the 503 504 contribution of each fraction to biodegradability was investigated. As model N_{vs} was not validated in the previous section, only normalized coefficients associated with models N_{EPS_G1} and for N_{EPS_G2} are 505 considered in this section and are presented in Figure 5. Errors bars associated to the normalized 506 507 coefficients in figure 5 are from uncertainty estimation considering 95 % confidence interval. The 508 impact of each fraction is considered significant if the obtained confidence interval does not contain 0. For both models, the fractions that had the most significant impact on nitrogen biodegradability were 509 NSOL EPS and NHOLO EPS. This result is in agreement with the organic fractions targeted by this 510 511 fractionation method. Indeed, PROT_EPS targets the protein present in extrapolymeric substances 512 (Jimenez et al., 2015) which are recalcitrant to biodegradation. Neither does NLIGN EPS contribute significantly, due to the strongly limited bioaccessibility of the compounds contained in this fraction. 513 In comparison with a similar study investigating the link between EPS fractionation and carbon 514 515 biodegradability, our results corroborate the observation made by Jimenez et al. (2017) concerning the 516 contribution of the non-extractible fraction (LIGN EPS). Indeed, LIGN EPS on carbon did not 517 contribute to the model like NLIGN_EPS to nitrogen based models. However, the extractible fractions 518 of organic nitrogen (NSOL_EPS, NPROT_EPS and NHOLO_EPS) do not contribute at the same level as organic carbon, which is explained by the difference in the distribution of organic nitrogen and 519 carbon discussed in section 3.1.2. 520

521	The main difference between N_{EPS_G1} and N_{EPS_G2} is the contribution of NPROT_EPS to nitrogen
522	biodegradability. Indeed, for G1 (substrates with $NH_4^+/NTK < 30\%$), this explanatory variable
523	contributes moderately to organic nitrogen biodegradability, but has no significant effect for G2
524	(substrates with $NH_4^+/NTK > 30\%$). This difference explains the difference in biodegradability of
525	organic nitrogen in the two groups of substrate by showing that for substrates that have a NH_4^+/NTK
526	ratio of less than 30%, part of the organic nitrogen contained in NPROT_EPS fraction is
527	biodegradable. However, for substrates with NH_4^+/NTK ratio greater than 30%, biodegradable organic
528	nitrogen contained in NPROT_EPS fraction was degraded before anaerobic digestion, i.e. during
529	fermentation which occurs during silageing, during animal digestion in the case of livestock manure or
530	during aerobic or anaerobic degradation processes that spontaneously occur during substrate storage.
531	Hence, for that substrate group, the organic nitrogen contained in the PROT_EPS fraction is not
532	significantly biodegradable under anaerobic digestion.
533	
534	4. CONCLUSION
535	Our results highlight the fact that the organic nitrogen in substrates is contained in fractions with
536	different bioaccessibility ranging from highly accessible to inaccessible. The distribution of organic
537	nitrogen depends to a great extent on the substrate. Therefore, like for carbon, organic nitrogen
538	biodegradability strongly depends on the substrate and can be predicted using statistical models that
539	use bioaccessibility indicators as variables. These models enable the fast determination of ammonium
540	and organic nitrogen in the digestate and help to optimize anaerobic digestion plants aimed at
541	advancing the substitution of mineral fertilizers by digestates and preventing ammonia inhibition.
542	
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640



Figure 1: Organic carbon (a) and organic nitrogen (b) distribution according to Van Soest and EPS
fractionation (Fraction abbreviations are explained table 2).



1).

- 649 Figure 2: Hierarchical Cluster Analysis of the panel of substrates (a) and (b) the distribution of the
- ratio NH4+/TKN according to the substrate typology (Substrate abbreviations are explained in table 650
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654 Figure 3: Box plots of the distribution of organic carbon and nitrogen biodegradability for G1 (a) and G2 (b).

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659 Figure 4: Organic carbon (a) and organic nitrogen (b) content for G1 and G2substrates according to 660 modified EPS fractionation (Fraction abbreviations are explained table 2).





Table 1: Characterization of the panel of substrates.

<u></u>		TS	VS	$\mathrm{NH_4}^+$	TKN	TOC	Protei
lass	Sample	$(g_{TS}.kg^{-1})$	$(g_{VS}.kg^{-1})$	$(gN .kg^{-1})$	(gN.kg ⁻¹)	(gC.kg ⁻¹)	(% _{org-}
residues	Faba beans	140.2	125.6	0	6.4	72.4	38%
	Vetch	174.5	158.6	0	8.4	86.5	42%
	Phacelia	216.6	197.7	0	2.8	49.9	24%
	Mustard	178.1	158.7	0	5.2	89.2	25%
	Oat	157.1	141.1	0	4.8	106.7	20%
	Rye	207.6	195.6	0	3.6	128.9	30%
	Barley	246.5	221.3	0	4.0	149.1	12%
	Corn silage	298.2	275.6	0.3	6.2	133.4	19%
	Grass silage	267.4	234.0	0.7	7.7	138.8	22%
	Oat silage	161.9	134.0	1.9	4.2	85.6	12%
ock manure	Cattle manure	133.7	103.8	1.3	4.3	71.0	18%
	Pig slurry	50.3	37.1	2.3	3.2	25.1	17%
	Cattle slurry	92.4	73.4	0.9	2.4	48.3	14%
	Poultry manure	328.1	252.2	5.5	13.8	148.4	24%
agroindustry and	Screening refusal from slaughterhouse (SRS)	294.1	282.7	0.2	7.8	165.4	20%
icipalities	Grass clippings (GC)	261.4	213.3	0.4	8.1	112.0	30%
	Cattle feed waste (CF)	698.1	628.1	3.4	34.0	321.9	41%
	Kitchen Waste (KW)	259.1	206.2	0.1	11.5	139.7	36%
	Waste from yeast production (ELVI)	587.7	465.0	4.9	43.9	251.3	67%
	Vegetable waste from supermarkets (SVW)	107.4	95.7	0.7	2.7	62.6	14%
667	0						
668	6						

Modifi	ed Van Soest method	Modified EPS method			
Fraction name**	Extraction step	Fraction name**	Extraction step		
SOL_VS	Water extraction	SOL_EPS	CaCl ₂ extraction		
PROT_VS	Neutral detergent extraction (NDF)	PROT_EPS	NaOH, NaCl 10mM + Na 0.1M extractions		
HOLO_VS	Acid detergent (ADF) + H ₂ SO ₄ 72% extraction	HOLO_EPS	H ₂ SO ₄ 72% extraction		
LIGN_VS	Non-extractible	LIGN_EPS	Non-extractible		

Table 2: Fractions and extractions steps in the modified Van Soest and EPS methods.

* In addition to targeted molecules, other compounds are also liberated by modifying accessibility.

671 ** If N prefix is added prior to the fraction name, organic nitrogen content in the fraction is considered,

9

672 otherwise, organic carbon content is considered.

Sec. 1	BMP	of CIT	Organic carbon bi	AM	
Sample Faba beans Vetch Phacelia Mustard Oat Rye Barley Corn silage Grass silage Oat silage Cattle manure Pig slurry Cattle slurry Poultry manure Screenings refusal from slaughterhouse (SRS) Grass clippings (GC) Cattle feed waste (CF) Kitchen waste (KW) Waste from yeast production (ELVI) Supermarket vegetable waste (SVW) 675 676	(Nm ³ .kgTS ⁻¹)	%СH ₄	gC.kgTS ⁻¹	% _{org-C}	gN-NH4 ⁺ .kgTS ⁻¹
Faba beans	267.9	53%	291.6	54%	21.3
Vetch	297.8	56%	289.1	58%	36.3
Phacelia	167.8	54%	171.0	74%	11.7
Mustard	241.7	45%	321.8	61%	19.7
Oat	325.2	52%	360.0	51%	19.1
Rye	301.0	44%	271.8	45%	5.4
Barley	272.5	44%	262.1	43%	4.4
Corn silage	220.9	45%	225.9	56%	3.1
Grass silage	320.5	46%	310.3	58%	13.3
Oat silage	365.9	60%	323.0	63%	2.2
Cattle manure	212.0	56%	196.4	36%	6.8
Pig slurry	235.3	70%	182.8	36%	3.4
Cattle slurry	251.9	64%	242.7	41%	3.7
Poultry manure	221.2	65%	182.8	41%	13.1
Screenings refusal from slaughterhouse (SRS)	299.2	60%	249.8	40%	6.0
Grass clippings (GC)	270.6	54%	230.4	54%	17.1
Cattle feed waste (CF)	396.6	65%	329.8	72%	35.7
Kitchen waste (KW)	220.9	54%	391.0	72%	20.4
Waste from yeast production (ELVI)	320.5	55%	248.1	58%	31.0
Supermarket vegetable waste (SVW)	365.9	41%	384.9	70%	7.6
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Table 3: Results of carbon and nitrogen biodegradability

Table 4: Statistical models for the prediction of organic nitrogen biodegradability considering the

678 whole set of substrates and associated quality parameters.

ractionation	Explanatory variables	Biodegradability (BD) equation		
method	Explanatory variables	Biodegradability (BD) equation	Q ²	
odified EPS	NSOL_EPS, NPROT_EPS, NHOLO_EPS, NLIGN_EPS	$BD(orgN_N_{EPS}) = -5.14 + 1.48*NSOL_EPS + 0.07*NPROT_EPS$ + 1.76*NHOLO_EPS + 0.12*NLIGN_EPS	0.736	
odified Van Soest	NSOL_VS, NPROT_VS, NHOLO_VS, NLIGN_VS	$BD(orgN_N_{VS}) = -1.91 + 0.80*NSOL_VS + 0.72*NPROT_VS$ + 0.44*NHOLO_VS - 0.178*NLIGN_VS	0.571	
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Table 5: Statistical models for the prediction of organic nitrogen biodegradability considering

substrate group G1 and G2 and and associated quality parameters.

	Fractionation method	Group	Biodegradability (BD) equation	Model quality
		considered		Q ²
	Modified EPS	C1	$BD(orgN_N_{EPS_{GI}}) = -4,22 + 1,43*NSOL_EPS + 0,84*NPROT_EPS + 1,22*NHOLO_EPS + 0.03*NLIGN_EPS$	0.802
	Modified Van Soest	01	$BD(orgN_N_{VS_GI}) = -1.94 + 0.89*NSOL_VS + 0.81*NPROT_VS + 0.24*NHOLO_VS - 0.59*NLIGN_VS$	0.551
2	Modified EPS		$BD(orgN_N_{EPS_G2}) = -2.78 + 1.29*NSOL_EPS - 0.07*NPROT_EPS + 1.14*NHOLO_EPS + 0.19*NLIGN_EPS$	0.771
	Modified Van Soest	G2	$BD(orgN_N_{VS_G2}) = -3.32 + 0.56*NSOL_VS + 0.28*NPROT_VS + 0.63*NHOLO_VS + 0.27*NLIGN_VS$	0.057
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685	Table 6: Comparison of organic nitrogen biodegradability values obtained from experiments and
686	models.

			Livesto	ck feed waste			01	1 (CO)		·1 (CO)
				(G1)	Û	at (GI)	Cattle	slurry (G2)	Oat s	silage (G2)
Mod			Predic		Predic		Predic		Predic	
el			tion	Prediction	tion	Prediction	tion	Prediction	tion	Prediction
used	Q²	R²	(g/kg	error (%)	(g/kg	error (%)	(g/kg	error (%)	(g/kg	error (%)
			TS ⁻¹)		TS ⁻¹)		TS ⁻¹)		TS ⁻¹)	
N _{EPS}	0.7 36	0.7 89	34.1	5%	15.2	20%	2.4	37%	1.8	19%
N _{EPS} _G1	0.8 02	0.8 43	35.7	2%	18.7	2%	n.t.	n.t.	n.t.	n.t.
N _{EPS}	0.7 71	0.9 43	n.t.	n.t.	n.t.	n.t.	3.9	5%	2.0	10%
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689 Highlights:

- Organic nitrogen is contained in materials with various bioaccessibility levels.
- Organic nitrogen bioaccessibility differs from organic carbon bioaccessibility.
- Organic nitrogen biodegradability cannot be estimated from carbon biodegradability.
- Van Soest fractionation does not predict organic nitrogen biodegradability.

• Models using bioaccessibility data from EPS fractionation show good predictability.