

Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: A bioaccessibility approach

Y. Bareha, R. Girault, Julie Jimenez, Anne Tremier

▶ **To cite this version:**

Y. Bareha, R. Girault, Julie Jimenez, Anne Tremier. Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: A bioaccessibility approach. Bioresource Technology, 2018, 263, pp.425 - 436. 10.1016/j.biortech.2018.04.085 . hal-01829450

HAL Id: hal-01829450

https://univ-rennes.hal.science/hal-01829450

Submitted on 4 Jul 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ACCEPTED MANUSCRIPT

Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: a bioaccessibility approach.

Y. Bareha[1,2,3], R. Girault[1,3], J. Jimenez[4] and A. Trémier[1,3]

[1] Irstea, UR OPAALE, 17 av. de Cucillé, CS 64427, F-35044 Rennes, France

(E-mail : romain.girault@irstea.fr)

[2] Université Rennes 1, 2 rue du Thabor, CS 46510, 35065 Rennes Cedex, France

[3] Université Bretagne Loire, France

[4] INRA, UR50, Laboratoire de Biotechnologie de l'Environnement, F-11100 Narbonne, France

Abstract

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 organic nitrogen biodegradability and to investigate how it can be predicted from carbon biodegradability, and nitrogen bioaccessibility, respectively. Bioaccessibility was assessed using fractionation methods based on sequential extractions. Results showed that organic nitrogen was present in fractions whose bioaccessibility levels differed. Organic nitrogen and carbon biodegradability were also determined and compared. Results highlighted two groups of substrates: the first with an initial NH_4^+/TKN<30%, whose carbon and nitrogen biodegradability are similar; the second with an initial NH_4^+/TKN>30%, whose carbon and nitrogen biodegradability differ significantly. To enable prediction on all substrates, partial least square (PLS) regressions were carried out to link organic nitrogen bioaccessibility indicators to biodegradability. The models successfully predicted organic nitrogen biodegradability with a maximum prediction error of 10%.

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

ACCEPTED MANUSCRIPT

1. INTRODUCTION

Anaerobic digestion is currently attracting considerable interest for the management of agricultural 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 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 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, composting, etc.) with the aim of replacing mineral fertilizers. Indeed, anaerobic digestion results in the mineralization of part of the organic nitrogen into ammonium (Möller and Müller, 2012) , enabling 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 chain is subject to nitrogen leakages in the form of ammonia emissions during storage (Sommer, 1997) and after spreading on the land, or through nitrate leaching (Möller, 2015). In addition to their 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 and to optimize nitrogen uptake by crops, the anaerobic digestion process has to be designed taking the characteristics of the digestate into account, i.e. ammonia content and organic nitrogen content, 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 substrates is required.

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

ACCEPTED MANUSCRIPT

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 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 bioaccessibility of organic nitrogen.

Accessible organic matter refers to the compounds in the substrate that microorganisms can access for 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 fractionate organic matter according to its degree of bioaccessibility. Better characterization of the bioaccessibility of organic nitrogen in substrates will enable a better understanding of the variability of its anaerobic biodegradability. Methods using successive chemical extractions such as the Van Soest method, or more advanced methods can be used for this purpose.

The Van Soest fractionation method (Van Soest, 1963) is widely used to fractionate wastes including vegetables, sludges, and plants. It uses different solvents to extract soluble and lignocellulosic 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-like, hemicellulose-like and cellulose-like compounds, respectively. The residual organic matter is considered as lignin-like compounds. Many studies have explored the correlations between the Van 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 lignin+cellulose-like content and biodegradability of fruit and vegetables in anaerobic conditions. Despite these promising results, Mottet et al., (2010) pointed out that this fractionation method is too aggressive to be representative of biological disintegration that occurs during anaerobic digestion, which limits its use for the prediction of biodegradability. Jimenez et al., (2015) then developed a less 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 solutions, and targets protein-like substances; it ends with strong acid extraction targeting holocellulosic-like products. This fractionation, combined with 3D-fluorescence to assess the

ACCEPTED MANUSCRIPT

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., 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 BMP) to 3 days (for EPS fractionation). However, all these studies focused on the biodegradability of volatile solids or carbon compounds. Consequently, no data are available concerning the ability of these accessibility indicators, determined from EPS or Van Soest fractionation, to predict organic nitrogen biodegradability during anaerobic digestion.

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 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 using two fractionation methods. Next we compared the anaerobic biodegradability of nitrogen and carbon on the same panel of substrates. Finally, we performed PLS regressions to identify possible correlations between organic nitrogen biodegradability and bioaccessibility indicators provided by the two fractionation methods to determine which fractionation method better describes organic nitrogen biodegradability.

2. MATERIAL AND METHODS

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®).

ACCEPTED MANUSCRIPT

2.2. Physico-chemical characterization

Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN) and total ammonia nitrogen were measured using standard methods (APHA, 2005). TKN and ammonia concentrations were determined by distillation (TKN after mineralization). Total carbon and total nitrogen were measured in finely ground raw samples by elemental analysis according to the manufacturer's instructions (Thermoflash 2000, Thermoscientific®). Inorganic carbon in the raw samples was determined by calcination at 480 °C and elemental analysis of the ashes. Total organic carbon (TOC) was determined by subtracting inorganic carbon (IC) from total carbon (TC). Protein content in organic carbon was calculated using the following the equation (1):

(1) Protein = $((TKN\text{-}NH_4^+)*C/N_{protein})/TOC$

Where:

- Protein: protein content ($\%_{org\text{-}C}$).
- $C/N_{protein}$: carbon/nitrogen ratio equal to 4.348 $gC.gN^{-1}$ based on Ramsay and Pullammanappallil (2001).

2.3. Organic matter fractionation

The fractionation methods used in this study to characterize organic matter were based on sequential chemical extractions. The successive steps of the two fractionation methods, derived from EPS (Jimenez et al., 2015) and Van Soest (Van Soest, 1963), are described below. Four fractions were extracted with each method and presented similarities in terms of molecules targeted by the extracting solvents as presented in table 2, which made it possible to compare the two methods. Uncertainty on fractionation results was estimated for each extraction method using a Monte Carlo method in order to take error propagation into account.

2.3.1. *Modified Van Soest fractionation*

The modified Van Soest fractionation is based on successive extractions with neutral and acid detergents and ending with a strong acid extraction. Organic matter was separated into four fractions:

ACCEPTED MANUSCRIPT

- SOL_VS: This fraction is extracted in water at a temperature of 20 °C for 1 h. The extraction temperature is different from that described in the extraction protocol for the French standard XPU 44-162 (AFNOR) where extraction is performed at 100 °C.
- PROT_VS : This fraction is extracted with boiling commercial neutral detergent (NDF: 1.86% disodium ethylene diamine tetraacetate, 3% aqueous solution of sodium dodecyl sulfate, 0.68% decahydrate sodium tetraborate, 0.45% disodium hydrogen phosphate and 1.0% triethylene glycol; Carlo Erba®) for 1 h from the pellet obtained after the SOL_VS step.
- HOLO_VS: This fraction results from the two successive extractions of the Van Soest protocol: (1) extraction with boiling commercial acid detergent ADF (2.6% sulfuric acid and 2.0% cetrimonium bromide; Carlo Erba®) for 1 h, followed by (2) extraction with 72% sulfuric acid (w/w) at ambient temperature for 3 h. This step used the pellet remaining from the PROT_VS step.
- LIGN_VS: This fraction contained the remaining materials not extracted during the previous extractions.

After the first extraction, the mix was centrifuged at 12100 g for 20 minutes. The supernatant containing the soluble fraction of organic matter was removed and the remaining pellet was then dried at 40°C and then ground to 1 mm. Detergent and strong acid extraction (PROT_VS and HOLO_VS steps) were performed on 1 gram of the dried-ground sample sealed in filter bags (F57 Filter Bags, Ankom Technologies®). Detergent and acid extractions were performed using an automated fiber analyzer (Ankom 2000, Ankom Technologies®). After each extraction step, the content of one bag was used to analyze total organic carbon and total remaining nitrogen using an elemental analyzer (Thermoflash 2000, Thermoscientific®). The organic carbon and nitrogen content in each fraction were determined by the difference between the organic carbon and nitrogen content in the filter bags before and after each extraction.

2.3.2. Modified "EPS" fractionation

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

ACCEPTED MANUSCRIPT

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 $CaCl_2$ 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 H_2SO_4 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.

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

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

ACCEPTED MANUSCRIPT

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 apparent organic nitrogen mineralized during anaerobic digestion. This method allows the determination of the organic nitrogen part of the substrate converted into soluble ammonium which is 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 monitored during the test. However, the inoculum used for ANMP tests was previously centrifuged at 12100 g for 20 minutes at ambient temperature and the supernatant was removed in order to reduce the initial ammonium concentration in the batch reactors. The remaining pellet was suspended in a $KHCO_3$ 10 g.L^{-1} + $NaHCO_3$ 10 g.L^{-1} solution to preserve sufficient buffering capacity in the batch and to prevent inhibitions due to acidification during incubation. A blank experiment was performed without the addition of substrate to assess the apparent organic nitrogen mineralization due to the 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 equation (2).

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

Where:

- AMNP : Apparent Nitrogen Mineralized Potential of the substrate (gN-NH_4^+.kgTS$_{substrate}^{-1}$)
- $NH_{4-substrate}^{+}$: total mass of ammonium into the batch experiment with substrate after incubation (gN-NH_4^+)
- $NH_{4-blank}^{+}$: total mass of ammonium into the blank batch experiment without substrate after incubation (gN-NH_4^+)
- $MV_{substrate-added}$: total mass of the volatile matter of the substrate added to the batch experiment (kgTS$_{substrate}$)

ACCEPTED MANUSCRIPT

- $NH_{4-Initial\ substrate}^{+}$: Ammonium concentration in the substrate before incubation (gN-NH_4^+.kg$TS_{substrate}^{-1}$).

Ammonium and TKN contents were measured before and after each incubation test using the distillation method to check nitrogen mass balance. The ammonium distillation method is aggressive 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 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%.

ANMP is an apparent mineralization potential which considers organic nitrogen from substrate mineralization and ammonium immobilization for biomass growth. To quantify the impact of ammonium immobilization for biomass growth on ANMP value, a control batch experiment was performed following the ANMP protocol with a volatile fatty acid solution as substrate. This experiment highlights an immobilization rate of 0.006 gN-$NH_4^+{}_{immobilized}$.$gC_{degraded}^{-1}$. This value is lower than the references found in the literature that range from 0.017 to 0.009 gN-$NH_4^+{}_{immobilized}$.$gC_{degraded}^{-1}$ (Batstone et al., 2002; Ramsay and Pullammanappallil, 2001).However, these constants only consider growth, while in this study, the constant is the result of both growth and partial decay during 40 days. This constant was applied to the panel of substrate. Hence, 6 ±3% of the TKN brought by the substrate can be immobilized for biomass growth after 40 days of incubation.

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

2.5. Statistical analysis

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

ACCEPTED MANUSCRIPT

Models based on fractionation methods were developed to describe and predict organic nitrogen biodegradability using PLS regression. PLS regression is an extended multiple linear regression that include principal component analysis principles in the regression structure. This technique build model to link one dependent variable to different explanatory variables by minimizing their covariance. Here, the dependent variable (data to predict) was organic nitrogen biodegradability ($gN\text{-}NH_4^+.kgTS^{-1}$) and 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 EPS fractionation (expressed in $gN.kgTS^{-1}$). In this study, model robustness was investigated using the following quality parameters:

- The coefficient of determination R^2, which is the proportion of the dependent variable variance that can be explained by the explanatory variable set and varies between 0 and 1. Based on previous studies using PLS for biodegradability prediction (Jimenez et al., 2017), a coefficient of determination superior to 0.8 is required to ensure a good fitting of the experimental data.
- The cross validated coefficient of determination Q^2, which is the variation rate of the dependent variable predicted by the model by cross validation. Q^2 makes it possible to judge 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 Q^2 value above 0.5 indicates good predictability of the model (Jimenez et al., 2014). Cross-validation was done using the jackknife method by excluding 1 out of 4 values in the data set chosen at random.

External model validation was done by applying the models to another set of data covering a wide range of biodegradability and substrate origin. The predicted organic nitrogen biodegradability was compared with the measured values.

ACCEPTED MANUSCRIPT

3. RESULTS AND DISCUSSION

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).

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 considerably from one substrate to another, with SOL_VS ranging from 13%$_{TOC}$ to 48%$_{TOC}$, PROT_VS from 5%$_{TOC}$ to 37%$_{TOC}$ and HOLO_VS from 9%$_{TOC}$ to 66%$_{TOC}$. Only LIGN_VS showed 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, organic nitrogen distribution shows that nitrogen was present in all fractions and, like carbon, is strongly substrate dependent. Indeed, the ranges of nitrogen contents were wide in each fraction. On average, only about 30%$_{org\text{-}N}$ was contained in the NPROT_VS fraction. The remaining organic nitrogen was mainly spilt between the NSOL_VS (35%$_{org\text{-}N}$ on average) and NHOLO_VS fractions (30%$_{org\text{-}N}$ on average). The range of nitrogen contents was wide in all fractions except NLIGN_VS. Only a small proportion of organic nitrogen (between 1 and 15%$_{org\text{-}N}$) was contained in LIGN_VS. 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 before composting (Denes et al., 2015) or soil mineralization of organic waste (Chalhoub et al., 2013; Garnier et al., 2003).

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%$_{org\text{-}N}$ for NSOL_EPS, 3

ACCEPTED MANUSCRIPT

to 41%$_{org-N}$ for NPROT_EPS, 3 to 29%$_{org-N}$ for NHOLO_EPS and from 17 to 67%$_{org-N}$ for NLIGN_EPS. The wide range of variability obtained shows that the carbon distribution in the EPS fractions is strongly substrate dependent. In addition, organic carbon and nitrogen are largely present in the non-extractible fraction LIGN_EPS and NLIGN_EPS reflecting that the extraction method uses 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 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 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, 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. Consequently, this fractionation method not only takes into account the nature of the extracted molecules, but also their bioaccessibility due to physical structure.

3.1.3. *Comparison of carbon and nitrogen distribution obtained with the modified Van Soest and modified EPS fractionation methods*

The distributions of organic carbon and nitrogen obtained with modified EPS and modified Van Soest 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 fractionation: HOLO_VS was 36%$_{TOC}$ higher than HOLO_EPS and PROT_VS was 66%$_{TOC}$ higher than PROT_EPS. In contrast, the non-accessible fraction was noticeably higher with the modified EPS method than with the modified Van Soest method. The LIGN_EPS fraction reached 41%$_{TOC}$ on average while LIGN_VS represented only 6%$_{TOC}$. The same kinds of results were obtained for organic nitrogen fractionation even if the trend of distribution between extractible fractions differed significantly. These observations show that a significant part of the organic carbon and nitrogen extracted with the Van Soest method solvents was not extracted by EPS solvents but remained in the non-extractible fraction LIGN_EPS.

ACCEPTED MANUSCRIPT

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 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 chemical composition on bioaccessibility (the ability of exoenzymes to reach active sites on organic compounds considering its chemical complexity but with no limitation caused by the physical 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 Soest fractionation is a detergent and results in strong disintegration of complex structures composed by lignin and holocellulose. Thus, this extraction step allows the extraction of physically inaccessible 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 fractionation did not disintegrate the organic matter and extracted only the physically and chemically accessible compounds. These results indicate that, on average, 38%$_{org\text{-}N}$ of the organic nitrogen and 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 containing lignocellulose compounds which strongly limits its bioaccessibility.

3.2. Organic carbon and nitrogen biodegradability under anaerobic conditions

3.2.1. Influence of substrate origin on biodegradability

Organic nitrogen biodegradability and carbon biodegradability were determined using the AMNP test and the BMP test. The results are presented in table 3.

Organic nitrogen biodegradability is strongly substrate dependent, ranging from 14%$_{org\text{-}N}$ of degradation to 82%$_{org\text{-}N}$ in the substrate panel, with an average value of 48%$_{org\text{-}N}$. In comparison, organic carbon biodegradability varied less, from 36 to 74%$_{TOC}$, with a mean value of 54%. 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

ACCEPTED MANUSCRIPT

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

To further investigate the variability of organic nitrogen and carbon biodegradability, substrate categories can be defined using a statistical method. To this end, three indicators were used that include the biodegradation rate for carbon and nitrogen, respectively, along with the NH_4^+/TKN ratio in the raw substrate. Hierarchical cluster analysis was performed on these variables for all substrates. The dendrogram (fig. 2a) highlights two groups of substrates based on the variables.

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

As highlighted in figure 3a, the first group G1 is mainly composed of substrates that present a slight difference between organic carbon biodegradation and organic nitrogen biodegradation with high 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 agro-industrial wastes and one silage sample out of three. As the biodegradability values for organic carbon and nitrogen are very close for these kinds of substrates, which contain less than 30% of 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 supermarket vegetable wastes as well as drain screenings from slaughterhouses with a percentage difference 39% and 37%, respectively between organic carbon and nitrogen biodegradability.

As shown in figures 2a, 2b and 3b, the second group, G2, is the opposite and is composed of substrates with a marked difference in the biodegradability of organic carbon and nitrogen, and with a high NH_4^+/TKN ratio (> 30%). Livestock manure is the most representative of this group but silage and stored agro-industrial waste show the same behavior. The biodegradation levels of these substrates containing large amounts of nitrogen in mineralized form are lower than for G1, with average carbon and nitrogen biodegradability of 43% and 30%, respectively. In addition, the biodegradability of organic carbon and nitrogen are not correlated in this type of substrate, making it impossible to accurately predict organic nitrogen biodegradability from the value obtained for organic carbon.

ACCEPTED MANUSCRIPT

382 The NH4+/TKN ratio indicates if organic nitrogen mineralization occurs before anaerobic digestion or
383 not. As highlighted by the results, these transformations can occur during long storage (supermarket
384 vegetable waste), animal digestion (livestock manures) or silage fermentation process (crop silage
385 samples). During these processes, carbon and nitrogen mineralization can differ. As an example,
386 during crop silaging, fermentation produces organic acids. These organic acids such as lactic acid lead
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
389 reaching mineralization and most of the BMP is preserved. However, as highlighted by Teixeira
390 Franco et al. (2016), the proteolytic activity can continue and a large amount of ammonia can be
391 produced indicating a significant organic nitrogen mineralization. This process can significantly
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
394 storage can have a differential impact on BMP and ANMP values. Therefore, the prediction of organic
395 nitrogen biodegradability from the value obtained for organic carbon (derived from BMP values) is
396 not relevant for most of the substrates belonging to the second group G2. Hence, depending on
397 transformations that can occur before anaerobic digestion, a substrate can move from G1 (fresh
398 substrates) to G2. In the results, this phenomenon is observed for oat that belongs to G1 after harvest
399 and to G2 after silage fermentation process.
400 The links between substrate typology and substrate characteristics (protein content, C/N ratio, organic
401 nitrogen fractionation following modified Van Soest and EPS methods) were investigated. C/N ratio,
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%$_{org\text{-}N}$ in average) is significantly inferior to the average value obtained for G1
408 (27%$_{org\text{-}N}$). In addition, the relative part of NLIGN_EPS for G2 (51%$_{org\text{-}N}$ in average) is higher than
409 for G1 (39%$_{org\text{-}N}$ in average). Differences between G1 and G2 are less significant for NPROT_EPS and

ACCEPTED MANUSCRIPT

NHOLO_EPS fractions. These results highlight that for substrates belonging to G2, transformations prior to anaerobic digestion (silage fermentation, storage or animal digestion) led to the mineralization of most of the bioaccessible organic nitrogen (NSOL_EPS fraction). Resulting substrates contain more hardly bioaccessible organic nitrogen (NLIGN_EPS fraction). These changes in bioaccessibility of organic nitrogen explain why average ANMP for G2 is significantly inferior to the average value obtained for G1. In addition, results highlight that the impact of transformations taking place before anaerobic digestion (silage fermentation, storage or animal digestion) impact differently organic carbon and nitrogen bioaccessibility, which can explain why prediction of organic nitrogen biodegradability from the value obtained for carbon is impossible for G2. These results highlight that the modified EPS fractionation allowed the determination of accessibility indicators which can explain biodegradability variations for organic nitrogen. Thus, using a fractionation method and especially modified EPS fractionation could help predict organic nitrogen biodegradability.

3.3. Prediction of organic nitrogen biodegradability from bioaccessibility indicators

3.3.1. Partial least squares (PLS) regression for the prediction of organic nitrogen biodegradability

PLS analysis was first carried out on the whole set of substrates. Secondly, the same analysis was performed on previously determined substrate groups (G1 end G2, see section 3.2), respectively.

3.3.1.1. Development of the statistical model considering the whole substrate set

Two PLS analysis were carried out to investigate correlations between organic nitrogen biodegradability and the bioaccessibility indicators obtained with the modified Van Soest and modified EPS fractionations, respectively, considering the whole set of substrates. Two statistical models were obtained (see Table 4): Model N_{EPS}, which considers modified EPS fractions of organic nitrogen as explanatory variables, and model N_{VS}, which considers modified Van Soest ones as explanatory variables. Both models show good model structure according to the cross validated 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 proportion of the organic nitrogen biodegradability variance that can be explained by the

ACCEPTED MANUSCRIPT

bioaccessibility indicators is higher with the N_{EPS} model (R^2 = 0.789) than with the N_{VS} model (R^2 = 0.640). The N_{EPS} model using modified EPS fractionation thus enables better simulation of the variation in nitrogen biodegradability, but R^2 is inferior of 0.8 so taking into account the typology determined in section 3.2. could improve the predictability of the models.

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 < 30%) and G2 (ratio of NH_4^+/NTK > 30%) whose organic nitrogen mineralization patterns differ. PLS analyses were thus performed to take them into account. Models considering modified EPS fractions 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} (Q^2 = 0.736 and R^2 = 0.789). Hence, model N_{EPS_G1} describing the biodegradability of the organic 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 regression, N_{EPS_G1} and N_{EPS_G2} models have satisfying R^2 values (0.818 for Jimenez et al. (2017), 0.88 for Monlau et al. (2012) and 0.925 for Wold et al. (2001)). These results reveal that substrate typology enables better accuracy and predictability with models using EPS fractionation as explanatory variables.

Models considering modified Van Soest fractions as explanatory variables (N_{VS_G1} for G1 and N_{VS_G2} for G2, see Table 5) present no real improvement of the R^2 with 0.668 and 0.455 for respectively N_{VS_G1} and N_{VS_G2}. These results showed that the variance of organic nitrogen biodegradability is not well described considering the typology of substrate by models using modified Van Soest variables. Also, the cross-validation parameter Q^2 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 results indicate that the substrate typology does not improve the robustness of statistical models considering Van Soest variables. Consequently, prediction of organic nitrogen biodegradability with

ACCEPTED MANUSCRIPT

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).

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 biodegradability of four substrates not used for model development was investigated. Predicted organic nitrogen biodegradability and prediction errors were only determined for models that showed 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 as explanatory variables, the prediction errors of the N_{EPS} model that considers the whole set of substrates, range from 5% to 37%. These results are better than those obtained with models using 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 substrates, G1 and G2, produced much better results. The N_{EPS_G1} and N_{EPS_G2} models that predict organic nitrogen biodegradability for substrates belonging to the groupe G1 and G2 respectively, 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 EPS fractions as explanatory variables and G1 and G2 as validity domains.

The EPS fractionation method has been used to develop accurate models for BMP prediction on various substrates (Jimenez et al., 2017). These models were completed with complexity variables acquired with 3D fluorescence spectrometry and reach a R^2 of 0.818 and a $Q^2 = 0.689$. The performance of the models developed in this study were similar without considering complexity, 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 physical structure and complexity, but using 3D fluorescence spectrometry makes it possible to track

ACCEPTED MANUSCRIPT

494 the recalcitrant materials released in the different fractions. The samples in the present study were used
495 raw with only light pretreatment as described in section 2.3.1 which saved the structure of organic
496 matter, and only bioaccessible materials were released during the different extraction steps. These
497 results highlight the fact that EPS fractionation allows the determination of bioaccessibility indicators
498 that take the impact of physical structure of the substrate organic matter into account in its
499 biodegradability.
500
501 *3.3.1.4. Analysis of the impact of bioaccessibility indicators on nitrogen biodegradability*
502 The normalized coefficient of each variable of the model can be determined from the correlation
503 between the explanatory variable and the dependant variable, hence, based on these coefficients, the
504 contribution of each fraction to biodegradability was investigated. As model N_{VS} was not validated in
505 the previous section, only normalized coefficients associated with models N_{EPS_G1} and for N_{EPS_G2} are
506 considered in this section and are presented in Figure 5. Errors bars associated to the normalized
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.
509 For both models, the fractions that had the most significant impact on nitrogen biodegradability were
510 NSOL_EPS and NHOLO_EPS. This result is in agreement with the organic fractions targeted by this
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
513 significantly, due to the strongly limited bioaccessibility of the compounds contained in this fraction.
514 In comparison with a similar study investigating the link between EPS fractionation and carbon
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
519 as organic carbon, which is explained by the difference in the distribution of organic nitrogen and
520 carbon discussed in section 3.1.2.

ACCEPTED MANUSCRIPT

The main difference between N_{EPS_G1} and N_{EPS_G2} is the contribution of NPROT_EPS to nitrogen biodegradability. Indeed, for G1 (substrates with NH_4^+/NTK < 30%), this explanatory variable contributes moderately to organic nitrogen biodegradability, but has no significant effect for G2 (substrates with NH_4^+/NTK > 30%). This difference explains the difference in biodegradability of organic nitrogen in the two groups of substrate by showing that for substrates that have a NH_4^+/NTK ratio of less than 30%, part of the organic nitrogen contained in NPROT_EPS fraction is biodegradable. However, for substrates with NH_4^+/NTK ratio greater than 30%, biodegradable organic nitrogen contained in NPROT_EPS fraction was degraded before anaerobic digestion, i.e. during fermentation which occurs during silageing, during animal digestion in the case of livestock manure or during aerobic or anaerobic degradation processes that spontaneously occur during substrate storage. Hence, for that substrate group, the organic nitrogen contained in the PROT_EPS fraction is not significantly biodegradable under anaerobic digestion.

4. CONCLUSION

Our results highlight the fact that the organic nitrogen in substrates is contained in fractions with different bioaccessibility ranging from highly accessible to inaccessible. The distribution of organic nitrogen depends to a great extent on the substrate. Therefore, like for carbon, organic nitrogen biodegradability strongly depends on the substrate and can be predicted using statistical models that use bioaccessibility indicators as variables. These models enable the fast determination of ammonium and organic nitrogen in the digestate and help to optimize anaerobic digestion plants aimed at advancing the substitution of mineral fertilizers by digestates and preventing ammonia inhibition.

5. AKNOWLEDGMENTS

This study was part of the MéthaPolSol and Concept-dig projects which are supported by the French Environment and Energy Agency (ADEME).

ACCEPTED MANUSCRIPT

6. BIBLIOGRAPHY

Alburquerque, J.A., De la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M.P., 2012. Agricultural use of digestate for horticultural crop production and improvement of soil properties. Eur. J. Agron. 43, 119–128.

APHA, 2005. Standard methods for the examination of water and waste water, 21st edn.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. The IWA Anaerobic Digestion Model No 1(ADM 1). Water Sci. Technol. 45, 65–73.

Chalhoub, M., Garnier, P., Coquet, Y., Mary, B., Lafolie, F., Houot, S., 2013. Increased nitrogen availability in soil after repeated compost applications: Use of the PASTIS model to separate short and long-term effects. Soil Biol. Biochem. 65, 144–157. https://doi.org/10.1016/j.soilbio.2013.05.023

Denes, J., Tremier, A., Menasseri-Aubry, S., Walter, C., Gratteau, L., Barrington, S., 2015. Numerical simulation of organic waste aerobic biodegradation: A new way to correlate respiration kinetics and organic matter fractionation. Waste Manag. 36, 44–56. https://doi.org/10.1016/j.wasman.2014.11.013

Dulekgurgen, E., Dogruel, S., Karahan, O., Orhon, D., 2006. Size distribution of wastewater COD fractions as an index for biodegradability. Water Res. 40, 273–282. https://doi.org/10.1016/j.watres.2005.10.032

Fouda, S., von Tucher, S., Lichti, F., Schmidhalter, U., 2013. Nitrogen availability of various biogas residues applied to ryegrass. J. Plant Nutr. Soil Sci. 176, 572–584. https://doi.org/10.1002/jpln.201100233

Garnier, P., Néel, C., Aita, C., Recous, S., Lafolie, F., Mary, B., 2003. Modelling carbon and nitrogen dynamics in a bare soil with and without straw incorporation. Eur. J. Soil Sci. 54, 555–568. https://doi.org/10.1046/j.1365-2389.2003.00499.x

González-Fernández, C., García-Encina, P.A., 2009. Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass Bioenergy 33, 1065–1069.

ACCEPTED MANUSCRIPT

573 Gunaseelan, V.N., 2007. Regression models of ultimate methane yields of fruits and vegetable solid
574 wastes, sorghum and napiergrass on chemical composition. Bioresour. Technol. 98, 1270–
575 1277. https://doi.org/10.1016/j.biortech.2006.05.014
576 Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.-P., Houot, S., Patureau, D., 2015. A new organic
577 matter fractionation methodology for organic wastes: Bioaccessibility and complexity
578 characterization for treatment optimization. Bioresour. Technol. 194, 344–353.
579 https://doi.org/10.1016/j.biortech.2015.07.037
580 Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction
581 of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential
582 extractions with fluorescence spectroscopy: Towards ADM1 variables characterization. Water
583 Res. 50, 359–372. https://doi.org/10.1016/j.watres.2013.10.048
584 Jimenez, J., Lei, H., Steyer, J.-P., Houot, S., Patureau, D., 2017. Methane production and fertilizing
585 value of organic waste: Organic matter characterization for a better prediction of valorization
586 pathways. Bioresour. Technol. 241, 1012–1021.
587 https://doi.org/10.1016/j.biortech.2017.05.176
588 Karahan, Ö., Dogruel, S., Dulekgurgen, E., Orhon, D., 2008. COD fractionation of tannery
589 wastewaters—Particle size distribution, biodegradability and modeling. Water Res. 42, 1083–
590 1092. https://doi.org/10.1016/j.watres.2007.10.001
591 Kobayashi, T., Wu, Y.-P., Lu, Z.-J., Xu, K.-Q., 2014. Characterization of Anaerobic Degradability and
592 Kinetics of Harvested Submerged Aquatic Weeds Used for Nutrient Phytoremediation.
593 Energies 8, 304–318. https://doi.org/10.3390/en8010304
594 Kobayashi, T., Xu, K.-Q., Li, Y.-Y., Inamori, Y., 2012. Effect of sludge recirculation on
595 characteristics of hydrogen production in a two-stage hydrogen–methane fermentation process
596 treating food wastes. Int. J. Hydrog. Energy, XII International Symposium on Polymer
597 Electrolytes: New Materials for Application in Proton Exchange Membrane Fuel Cells 37,
598 5602–5611. https://doi.org/10.1016/j.ijhydene.2011.12.123
599 Liu, X., Bayard, R., Benbelkacem, H., Buffière, P., Gourdon, R., 2015. Evaluation of the correlations
600 between biodegradability of lignocellulosic feedstocks in anaerobic digestion process and their

ACCEPTED MANUSCRIPT

biochemical characteristics. Biomass Bioenergy 81, 534–543. https://doi.org/10.1016/j.biombioe.2015.06.021

Massé, D.I., Croteau, F., Masse, L., 2007. The fate of crop nutrients during digestion of swine manure in psychrophilic anaerobic sequencing batch reactors. Bioresour. Technol. 98, 2819–2823. https://doi.org/10.1016/j.biortech.2006.07.040

Möller, K., 2015. Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review. Agron. Sustain. Dev. 35, 1021–1041. https://doi.org/10.1007/s13593-015-0284-3

Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Eng. Life Sci. 12, 242–257. https://doi.org/10.1002/elsc.201100085

Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. Chem. Eng. J. 160, 488–496.

Muller, M., Jimenez, J., Antonini, M., Dudal, Y., Latrille, E., Vedrenne, F., Steyer, J.-P., Patureau, D., 2014. Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. Waste Manag. 34, 2572–2580. https://doi.org/10.1016/j.wasman.2014.07.028

Palmisano, A.C., Barlaz, M.A., 1996. Microbiology of Solid Waste. CRC Press.

Ramsay, I.R., Pullammanappallil, P.C., 2001. Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation 12, 247–257.

Sommer, S.G., 1997. Ammonia volatilization from farm tanks containing anaerobically digested animal slurry. Atmos. Environ. 31, 863–868. https://doi.org/10.1016/S1352-2310(96)00250-6

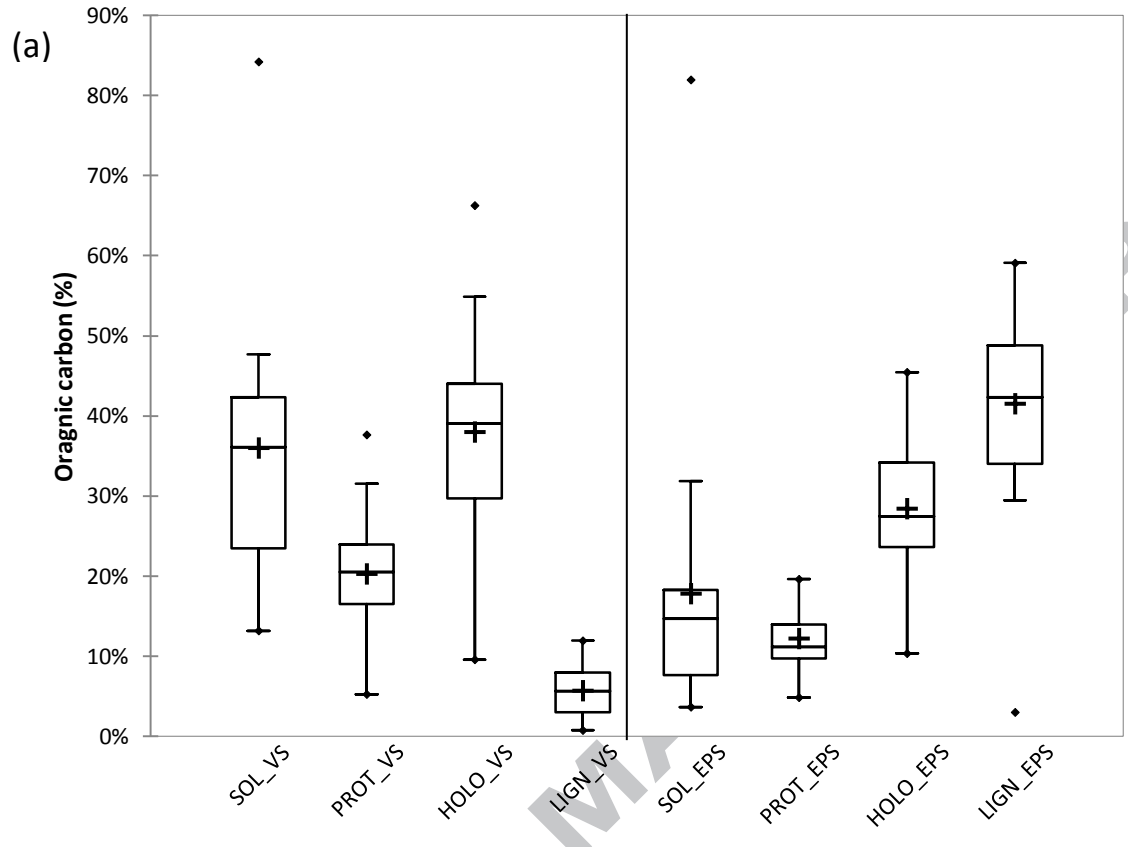
Tambone, F., Genevini, P., D'Imporzano, G., Adani, F., 2009. Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour. Technol. 100, 3140–3142. https://doi.org/10.1016/j.biortech.2009.02.012

ACCEPTED MANUSCRIPT

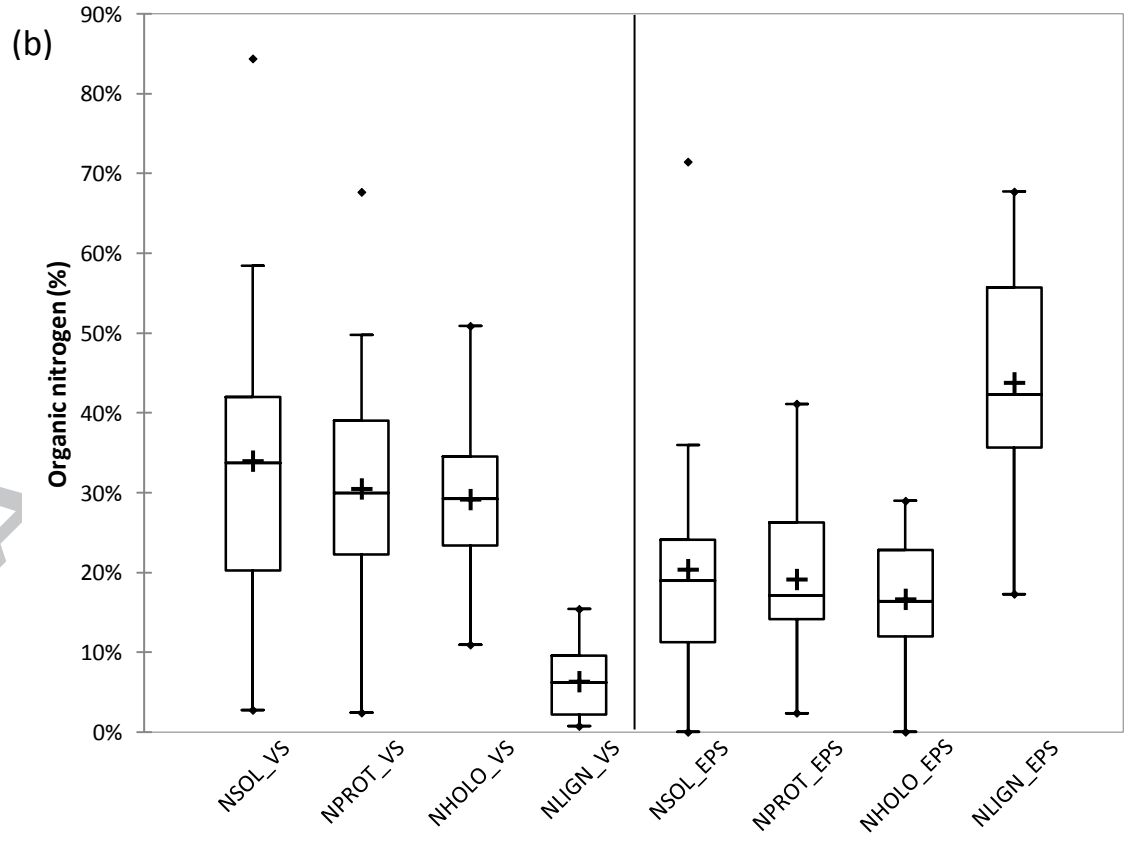
627 Teixeira Franco, R., Buffière, P., Bayard, R., 2016. Ensiling for biogas production: Critical
628 parameters. A review. Biomass Bioenergy 94, 94–104.
629 https://doi.org/10.1016/j.biombioe.2016.08.014
630 Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. 1. Preparation of fiber
631 residues of low nitrogen content. J. Assoc. Off. Agric. Chem. 46, 825–829.
632 Vedrenne, F., Béline, F., Dabert, P., Bernet, N., 2008. The effect of incubation conditions on the
633 laboratory measurement of the methane producing capacity of livestock wastes. Bioresour.
634 Technol. 99, 146–155. https://doi.org/10.1016/j.biortech.2006.11.043
635 Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemom.
636 Intell. Lab. Syst., PLS Methods 58, 109–130. https://doi.org/10.1016/S0169-7439(01)00155-1
637 Yang, G., Zhang, P., Zhang, G., Wang, Y., Yang, A., 2015. Degradation properties of protein and
638 carbohydrate during sludge anaerobic digestion. Bioresour. Technol. 192, 126–130.
639 https://doi.org/10.1016/j.biortech.2015.05.076
640
641

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



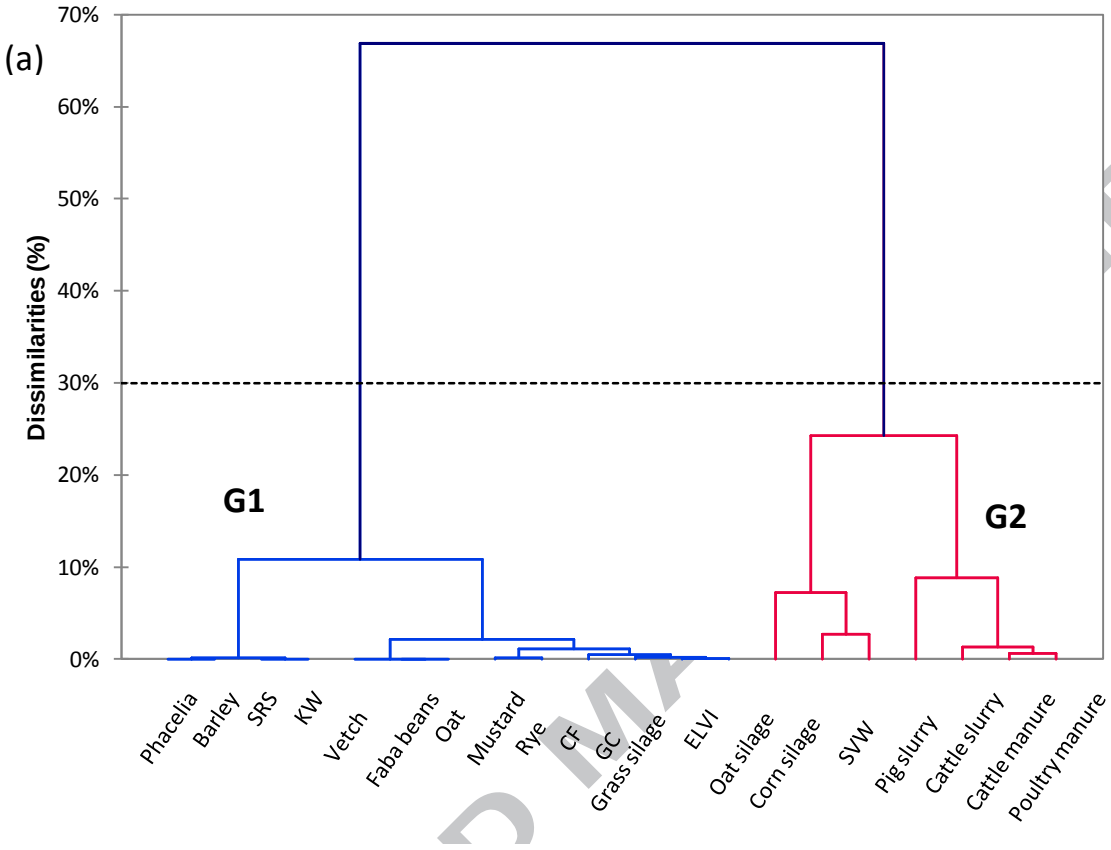
642



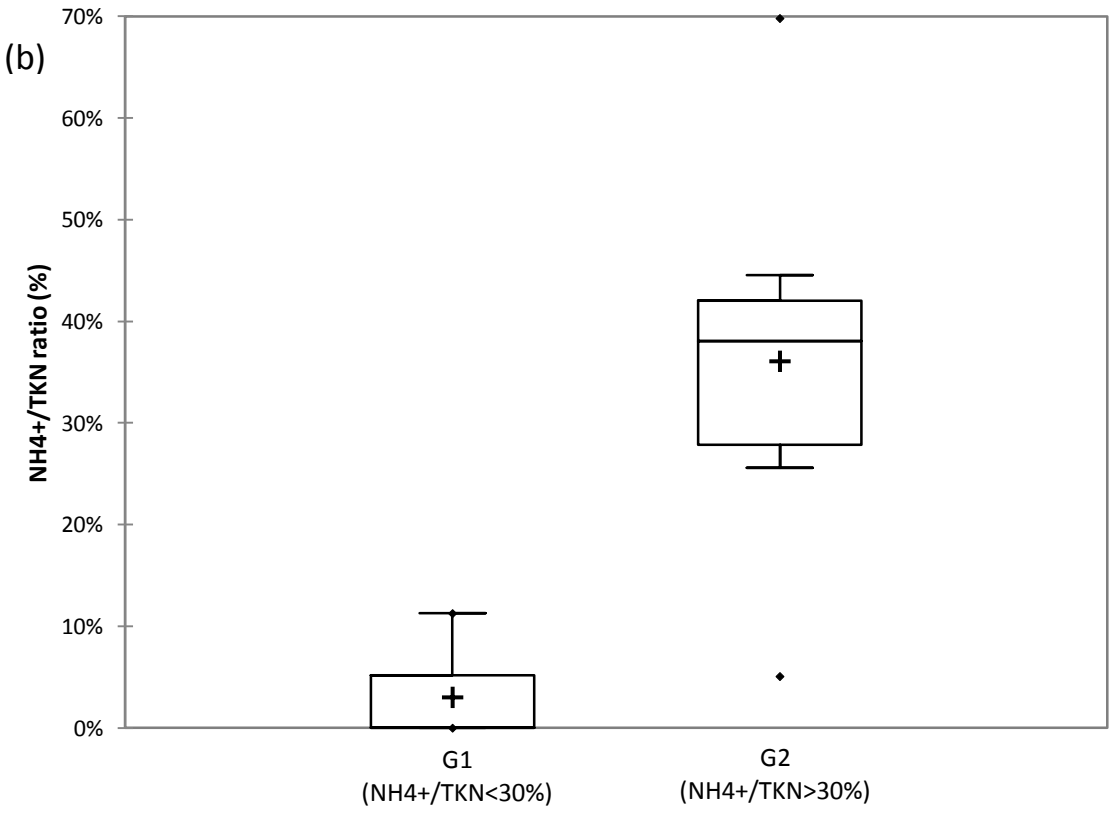
643

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

646


(a)
70%
60%
50%
40%
30%
20%
10%
0%
Dissimilarities (%)
G1
G2
Phacelia
Barley
SRS
KW
Vetch
Faba beans
Oat
Mustard
Rye
CF
GC
Grass silage
ELVI
Oat silage
Corn silage
SVW
Pig slurry
Cattle slurry
Cattle manure
Poultry manure

647


(b)
70%
60%
50%
40%
30%
20%
10%
0%
NH4+/TKN ratio (%)
G1
(NH4+/TKN<30%)
G2
(NH4+/TKN>30%)

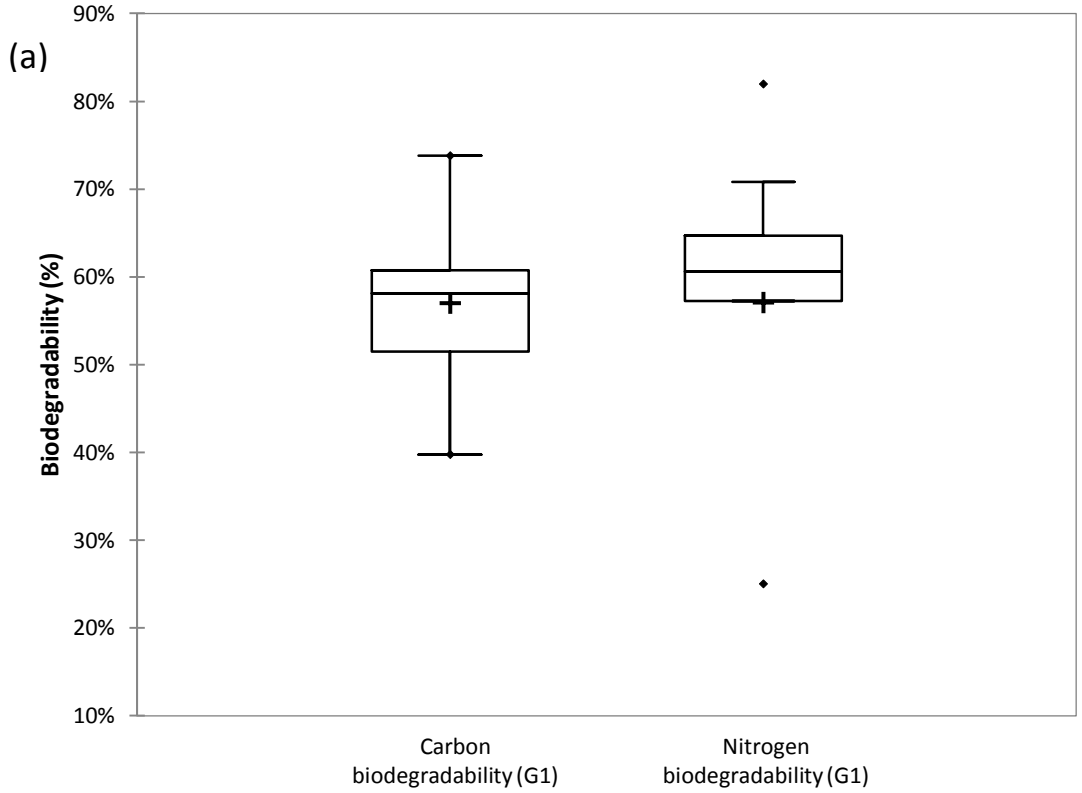
648

ACCEPTED MANUSCRIPT

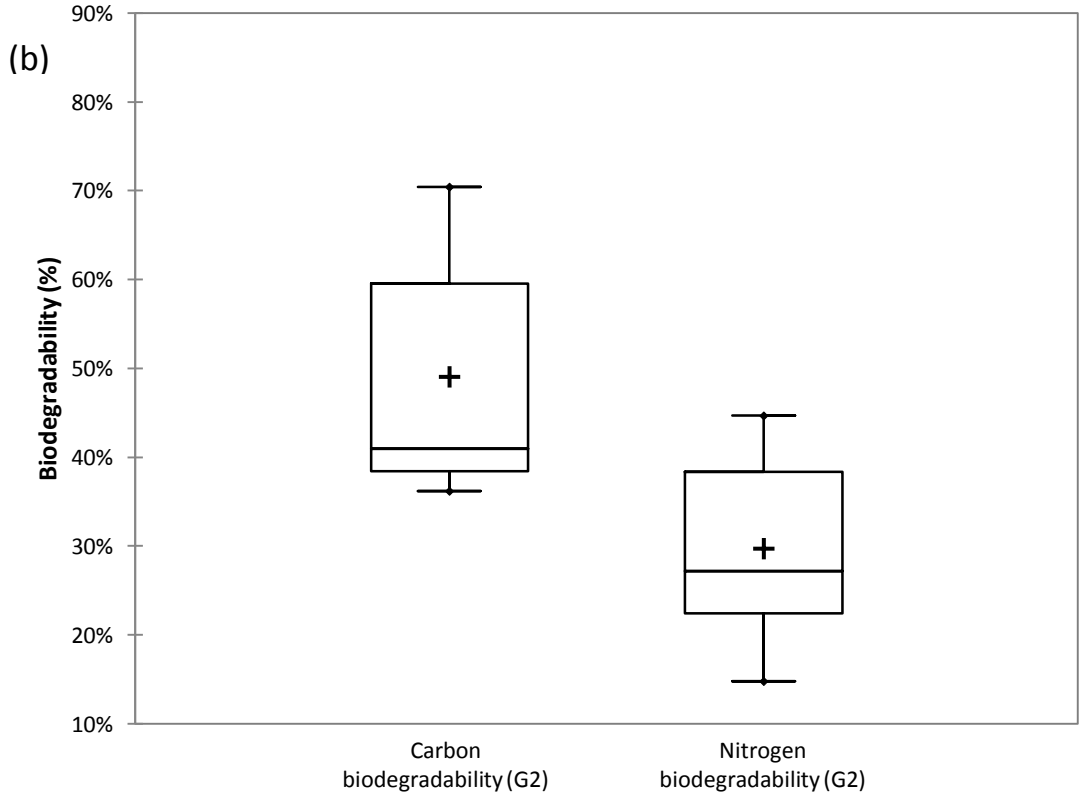
649 Figure 2: Hierarchical Cluster Analysis of the panel of substrates (a) and (b) the distribution of the
650 ratio NH4+/TKN according to the substrate typology (Substrate abbreviations are explained in table
651 1).

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



652



653

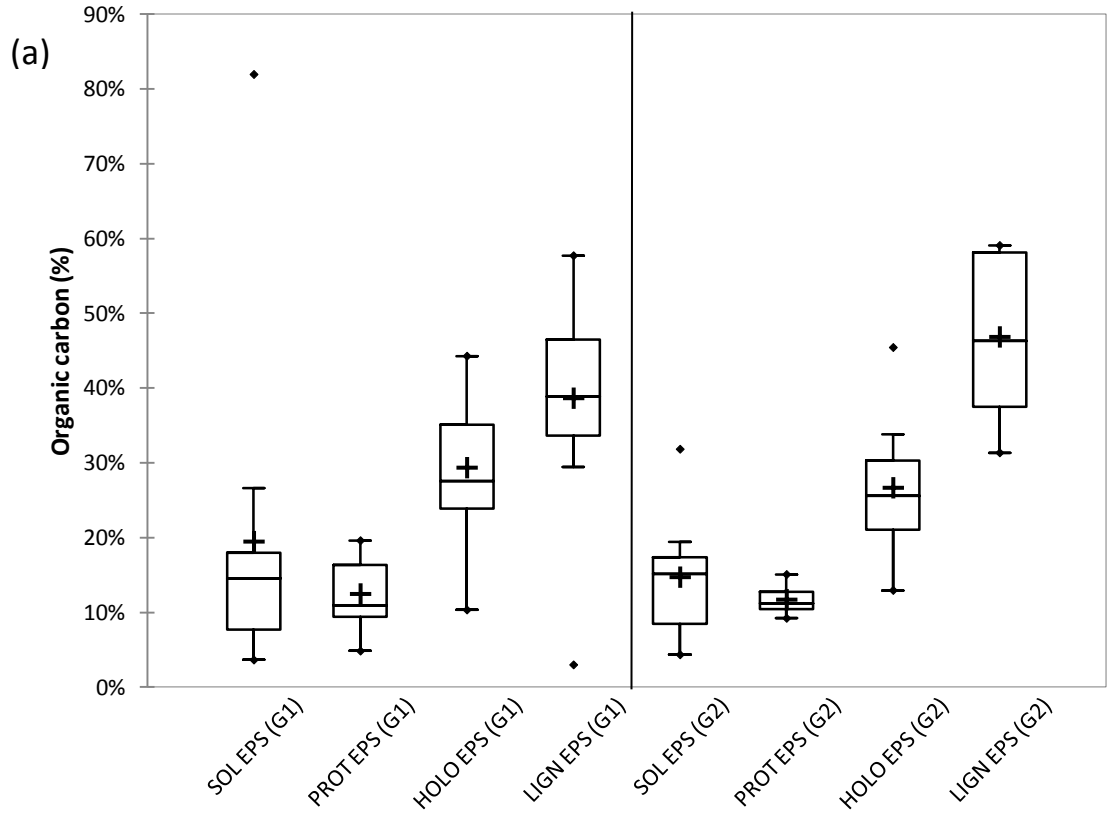
654 Figure 3: Box plots of the distribution of organic carbon and nitrogen biodegradability for G1 (a) and
655 G2 (b).

ACCEPTED MANUSCRIPT

656

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



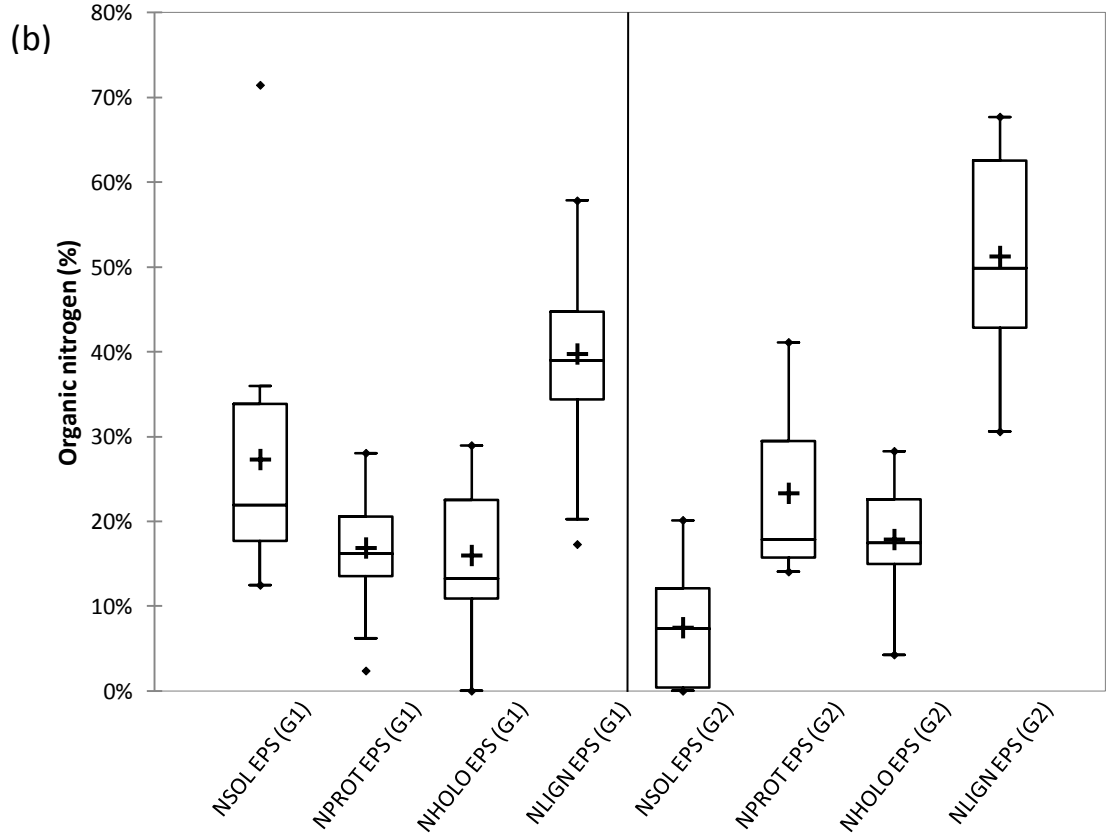
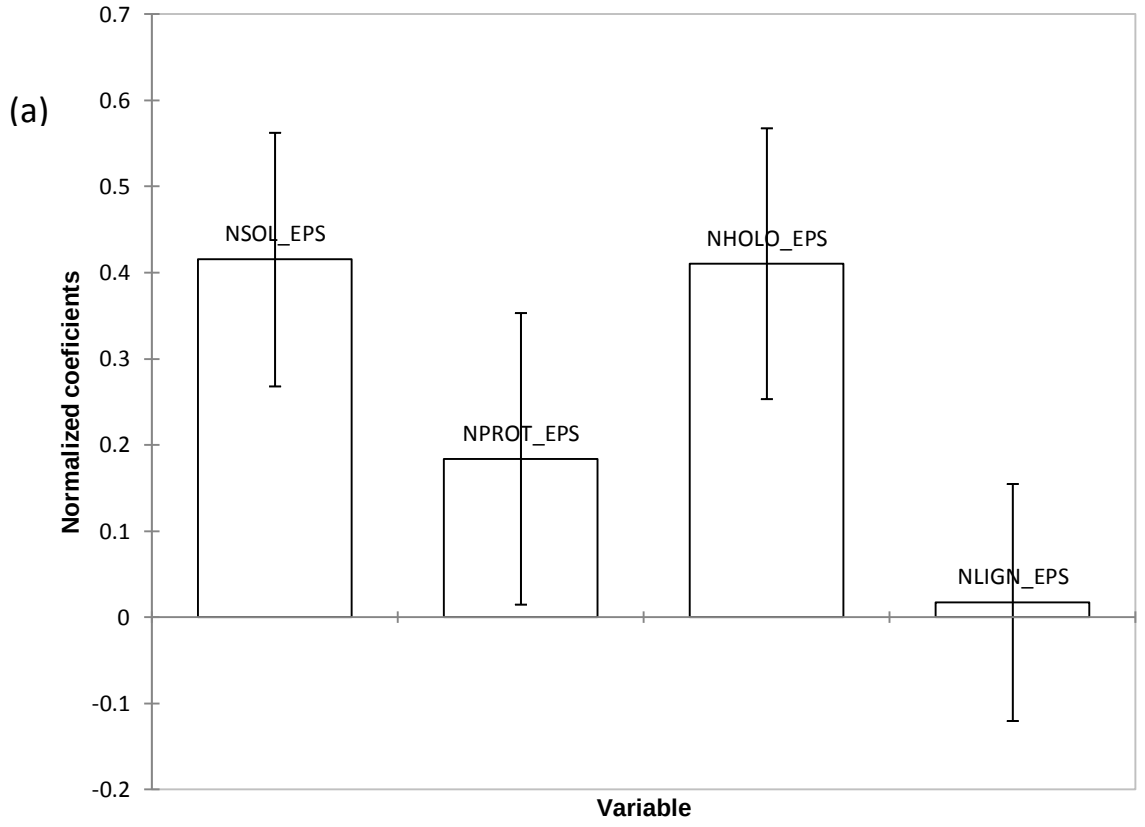
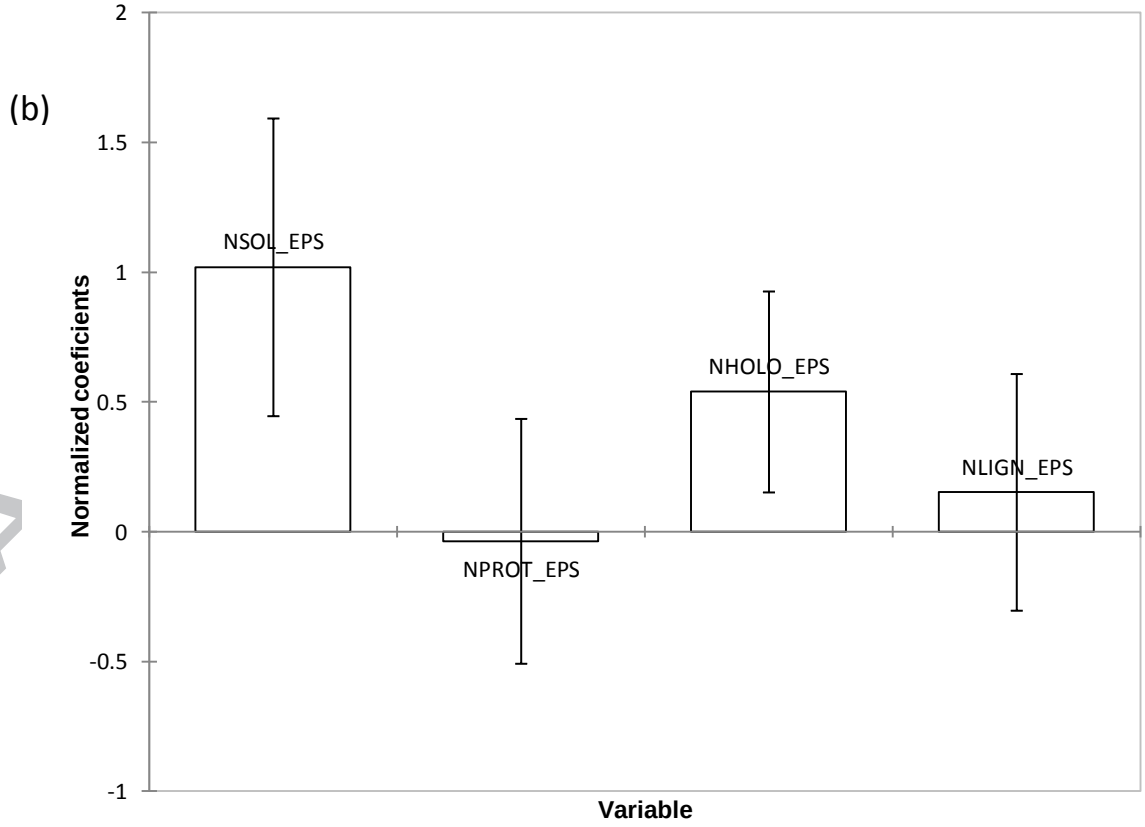


Figure 4: Organic carbon (a) and organic nitrogen (b) content for G1 and G2substrates according to modified EPS fractionation (Fraction abbreviations are explained table 2).

ACCEPTED MANUSCRIPT



661



662

663 Figure 5: Normalized coefficient for model N_{EPS_G1} (a) and N_{EPS_G2}(b) explaining organic nitrogen
664 biodegradation on G1 and on G2 (Fraction abbreviations are explained table 2).

ACCEPTED MANUSCRIPT

665

666 **Table 1:** Characterization of the panel of substrates.

Class	Sample	TS ($g_{TS}.kg^{-1}$)	VS ($g_{VS}.kg^{-1}$)	NH_4^+ ($gN.kg^{-1}$)	TKN ($gN.kg^{-1}$)	TOC ($gC.kg^{-1}$)	Protein ($\%_{org-C}$)
p 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

668

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

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

Modified 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_2SO_4 72% extraction	HOLO_EPS	H_2SO_4 72% extraction
LIGN_VS	Non-extractible	LIGN_EPS	Non-extractible

670 * 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,

672 otherwise, organic carbon content is considered.

673

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

674 **Table 3:** Results of carbon and nitrogen biodegradability

Sample	BMP ($Nm^3.kgTS^{-1}$)	$\%CH_4$	Organic carbon biodegradability		AM
			$gC.kgTS^{-1}$	$\%_{org-C}$	$gN\text{-}NH_4^+.kgTS^{-1}$
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

675

676

ACCEPTED MANUSCRIPT

677 **Table 4:** Statistical models for the prediction of organic nitrogen biodegradability considering the
678 whole set of substrates and associated quality parameters.

ractionation method	Explanatory variables	Biodegradability (BD) equation	Model qualit
			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

679

680

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

681 **Table 5:** Statistical models for the prediction of organic nitrogen biodegradability considering
682 substrate group G1 and G2 and and associated quality parameters.

Fractionation method	Group considered	Biodegradability (BD) equation	Model quality parameter
			Q^2
Modified EPS	G1	$BD(orgN_N_{EPS_G1})$ = -4,22 + 1,43*NSOL_EPS + 0,84*NPROT_EPS + 1,22*NHOLO_EPS + 0.03*NLIGN_EPS	0.802
Modified Van Soest		$BD(orgN_N_{VS_G1})$ = -1,94 + 0,89*NSOL_VS + 0,81*NPROT_VS + 0,24*NHOLO_VS - 0,59*NLIGN_VS	0.551
Modified EPS	G2	$BD(orgN_N_{EPS_G2})$ = -2.78 + 1.29*NSOL_EPS - 0.07*NPROT_EPS + 1.14*NHOLO_EPS + 0.19*NLIGN_EPS	0.771
Modified Van Soest		$BD(orgN_N_{VS_G2})$ = -3.32 + 0.56*NSOL_VS + 0.28*NPROT_VS + 0.63*NHOLO_VS + 0.27*NLIGN_VS	0.057

683

684

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

685 **Table 6:** Comparison of organic nitrogen biodegradability values obtained from experiments and
686 models.

Model used	Q^2	R^2	Livestock feed waste (G1)		Oat (G1)		Cattle slurry (G2)		Oat silage (G2)	
			Prediction (g/kg TS^{-1})	Prediction error (%)	Prediction (g/kg TS^{-1})	Prediction error (%)	Prediction (g/kg TS^{-1})	Prediction error (%)	Prediction (g/kg TS^{-1})	Prediction error (%)
N_{EPS}	0.736	0.789	34.1	5%	15.2	20%	2.4	37%	1.8	19%
N_{EPS_G1}	0.802	0.843	35.7	2%	18.7	2%	n.t.	n.t.	n.t.	n.t.
N_{EPS_G2}	0.771	0.943	n.t.	n.t.	n.t.	n.t.	3.9	5%	2.0	10%

687 n.t.: not tested

688

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

689 **Highlights:**

690 • Organic nitrogen is contained in materials with various bioaccessibility levels.

691 • Organic nitrogen bioaccessibility differs from organic carbon bioaccessibility.

692 • Organic nitrogen biodegradability cannot be estimated from carbon biodegradability.

693 • Van Soest fractionation does not predict organic nitrogen biodegradability.

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

695

ACCEPTED MANUSCRIPT