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Abstract

The aim of this work was to study an innovative alkaline process on two cover crops. CaO load of 60g.kgTS^{-1} was implemented to combine the functions of storage and pretreatment. Lab-scale reactors were monitored for 180 days to assess the effect of this process on the physico-chemical properties of the biomass. From the first days, pH was not maintained in an alkaline zone and microbial fermentation activity was observed with the degradation of available carbohydrates and production of metabolites, CO_2 and H_2. High butyric acid accumulation was observed and mass losses of 18.1% and 9.0% of initial VS occurred for oat and rye, respectively. However, no methane potential loss was recorded in the short and long term and the crops were efficiently preserved. The pretreatment had no major impact on fiber solubilization, and no increase in BMP was obtained, which was attributed to the short duration of the alkaline conditions.

Keywords

Biomethane; Biogas; Catch crops Storage; Pretreatment; Silage fermentation

1 Introduction

The production of biomethane by anaerobic digestion (AD) is likely to increase greatly in the coming years, as it is expected to replace part of the natural fossil gas. A large variety of organic wastes can be valorized such as sewage sludge, biowastes, manure and crop residues. Additionally, in order to supply a sufficient biomass to meet the demand for biogas, crops are also grown to feed agricultural biogas plants. These crops are interesting substrates for AD since they have a high CH_4 yield and allow a secure feed stock for the biogas plants. However, the cultivation of energy crops is in competition for arable land with food crops, which questions their

sustainability as a substrate for AD (Jury et al., 2010). Therefore, cover crops (CC), also called catch crops, are receiving an increasing interest as an alternative to energy crops. These crops are not being grown in place of food/feed crops, but during the intercultural period of their crop rotations. Thus, they avoid the bare soil period that may happen between two food crops cultivation. The use of cover crops in farming practices has both agricultural and environmental benefits, since it allows to avoid erosion and nutrient leaching (Igos et al., 2016; Jian et al., 2020; Sapkota et al., 2012), improves the accumulation of organic matter in the soil (Jian et al., 2020) and facilitates the control of undesirable weeds (Büchi et al., 2020). However, the use of CC for biogas production still faces some difficulties. First, the harvest only takes place once or twice a year, while the digesters are fed continuously. CC are often grown during short and unfavorable periods, and consequently their biomass yields are variable and unpredictable from one year to another. These two aspects make an efficient storage of the harvested crops a mandatory key point in their use for AD.

Currently, the storage step is performed by ensiling, a process widely used for forage storage for cattle feeding since the end of the 19th century (Goffart, 1877) . Ensiling relies on the spontaneous fermentation of the substrate under anaerobic conditions, allowing the release of organic acids (mostly lactic acid) and acidification of the medium (Elferink et al., 1999; Weinberg and Ashbell, 2003). The low pH under anaerobic conditions inhibits the microbial activity, thus ensuring the substrate stability over several months (Driehuis et al., 2003). When executed properly, ensiling allows a long-term preservation of the biomass energy potential, but various degradations may occur, depending on a large panel of parameters from the crop characteristics to the silo conception (Teixeira Franco et al., 2016). If this process has been optimized for animal feeding for

50 decades, its application to anaerobic digestion is still relatively new and may be improved. New
51 storing methods may also be developed, specifically designed for anaerobic digestion.

52 Another limit in the use of CC for AD is related to their physicochemical characteristics. The cell
53 walls are composed of a complex lignocellulosic matrix made of cellulose, hemicellulose and lignin.
54 This structure limits the access of the microorganisms to the highly degradable carbohydrates of
55 the cellulose and hemicellulose, lowering the yield and the kinetic of the crop conversion into
56 methane (Monlau et al., 2013b). In order to enhance the anaerobic digestion of such
57 lignocellulosic matrix, many types of pretreatment processes have been reported in the literature
58 and their actions on lignocellulose have been well described. Among the different technologies,
59 alkaline pretreatments are particularly relevant since alkaline conditions are highly effective in
60 solubilizing lignin (Carrere et al., 2016). High increases in biochemical methane potential (BMP)
61 were obtained by applying intensive conditions of pretreatments such as high temperature
62 (Sambusiti et al., 2012) or high chemical concentration (Jiang et al., 2017). In addition, significant
63 improvements in BMP were also observed in mild conditions, using NaOH, CaO or KOH, allowing
64 much lower energy consumption (Khor et al., 2015; Thomas et al., 2018). In these conditions, the
65 extension of the duration had a positive effect on the pretreatment effectiveness, and the
66 treatment duration appeared, under certain conditions, to be more favorable than the reactive
67 concentration (Thomas et al., 2018). Despite its lower efficiency when compared to other alkaline
68 compounds like NaOH and KOH, lime presents the advantage to be less expensive. Additionally,
69 CaO is already used on agricultural sites for soil liming to control acidity. Consequently, on an
70 agricultural biogas plant, the use of lime is much more compatible with the use of digestate as a
71 fertilizer than NaOH that causes an increase in the soil salinity due to sodium.

If alkaline pretreatments may constitute an interesting option for lignocellulosic biomass such as cover crops, applying them on stored silage does not constitute an adequate solution because of their high concentrations in acidic compounds such as lactic and acetic acids. A large amount of alkaline chemical would be necessary to neutralize the acids and increase the pH. For this reason, the implement of the alkaline pretreatment just after harvesting and extension of the pretreatment reaction time up to several months may constitute an alternative to ensiling for the storage of cover crops, while maximizing the alkaline treatment action. Compared to silage, which leads to a drop in pH in acidic regions (pH=4), microbial inhibition, which is necessary to maintain the methanogenic potential of the substrate, can be ensured by maintaining alkaline conditions.

However, as the addition of an alkaline agent is currently mostly used for pre-treatment process purposes and not for storage purposes, the duration of the treatment reported in the bibliography usually ranges from a few hours to 1 or 2 days and very few studies investigated longer duration of up to 7 days (Atelge et al., 2020). Digman et al. (2010) reported an alkaline pretreatment + anaerobic storage process of switchgrass and reed canarygrass using CaO for ethanol production. Several CaO loadings from 14.6 to 100g.kg^{-1} were applied with storage durations ranging from 30 to 180 days. The authors reported a good preservation of the substrate in all cases, even if no global energy balance including mass losses was calculated. In this study, the pretreatment efficiency increased together with lime loading and the highest ethanol yields were obtained with 85 and 100g.kg^{-1} CaO. However, the two studied solid content were very high (43 and 66%TS) and it was observed that moisture content had a positive effect on the pretreatment efficiency. This may suggest that interesting results with lower CaO loading can be obtained in wetter conditions. Finally, even if a correlation between the added CaO amount and the final pH of the silos was reported, no signs of undesirable fermentations were stressed. However, no dynamic monitoring

of the variation of the physico-chemical characteristics was made, making difficult the interpretation of the possible metabolic pathways that took place and fine understanding of the process.

The aim of this study is to evaluate the mechanisms involved during a long-term alkaline storage (i.e., CaO) of two cover crops, and its impact on the conservation/improvement of the energy potential in anaerobic digestion. The operating conditions were chosen to limit the pretreatment cost, following a procedure similar to ensiling, except the CaO addition as a dry powder before closing the silo. The conditions were set at ambient temperature. The main novelty of this work is the high solid content of the alkaline pretreatment, and the extended reaction duration until fulfilling the storage function. The dynamical changes in the physico-chemical characteristics of the substrate were observed over a six-month period as well as the impact on the methane potential.

2 Materials and Methods

2.1 Feedstock

Two cover crops were used for this experiment. An oat sample (*Avena sativa*) was collected on an experimental agricultural site (Arvalis, Montardon 64121, France) and a rye sample (*Secale cereale*) on another agricultural site (Biométharn, Aiguefonde 81200, France). Both crops were grown as winter cover crops, sowed in mid-October and harvested in the very last days of April at maturity stage BBCH 60 (beginning of flowering) and BBCH 59 (end of heading) for oat and rye, respectively. The crops were hand-harvested at approximate cutting height of 10 cm and stored overnight in sealed plastic bags as whole plants at 4°C before use.

2.2 Alkaline storage conditions

Prior to the experiment, the crops were chopped into 1-2 cm pieces using a garden shredder (AXT 2550TC, Bosch GmbH). Then, the alkaline reagent CaO was added as a dry powder and was homogenized manually. A CaO loading of 60g.kg_{TS}^{-1} was chosen as a compromise between pretreatment efficiency and reactive cost according to the literature. Jiang et al. (2017) found the most interesting lime load for 70 g.kg_{TS}^{-1} $Ca(OH)_2$ (equivalent to 53 g.kg_{TS}^{-1} CaO) while Thomas et al. (2018) and Khor et al. (2015) obtained significant BMP improvement for 50 g.kg_{TS}^{-1} CaO and 75 g.kg_{TS}^{-1} $Ca(OH)_2$ (equivalent to 57 g.kg_{TS}^{-1} CaO) respectively. The storage experiments were conducted in 2.6 L glass flasks sealed with air-tight lids equipped with a rubber septum that allows gas sampling, pressure measurement and pressure release. The experimental setup was inspired by the one used by Hillion (2017), which provided a high repeatability. For each crop, 5 replicates were prepared to be sacrificed after 2, 7, 21, 60 and 180 days to monitor the impact of the pretreatment on biomass conservation and the variation of the physico-chemical properties. 700 g of samples were introduced in each flask just after mixing with CaO, and packed manually until a density of 500 gFM.L^{-1}. The flasks were finally flushed with N_2, sealed and stored in a dark place at 22°C until their opening.

2.3 Silo monitoring and sampling

Gas production and mass losses were monitored regularly all over the experiment. The volumetric gas production was measured by a pressure difference method. Gas sampling and pressure release were made as often as necessary depending on the gas production kinetics. The pressure was measured through the septum with a manometer (Keller LEO 2). Gas was released when pressure exceeded 1.2 bar, and the flask was weighted. The volume of gas inside the flasks (headspace +

pore space) was calculated by subtracting the volume of added substrate to the flask volume. The volume of the substrate was calculated using the theoretical density equation adapted from McNulty *et al.* (1982) (Eq.(1)):

$$\rho_t = (1 - C)\,[(M/\rho_l) + (1 - M)/\rho_s] + C/\rho_{CaO} \quad (1)$$

where M = fractional moisture content related to fresh matter (FM); C = CaO content related to fresh matter; ρ_l = water density = 1000 kg.m^{-3}; ρ_s = dry matter density = 1421 kg.m^{-3} and ρ_{CaO} = CaO density = 3345 kg.m^{-3}. The gas composition was analyzed using a gas chromatography (Perkin Elmer Clarus 580) as described in section 2.4.

For each sampling date, one flask was opened after measuring final gas volume and composition and weight. The whole sample was homogenized and mixed using a knife mill (Pulverisette 11, Fritsch). Samples were prepared and stored for BMP test by freezing around 2 g_{TS} of matter at -20°C. Total solids (TS) and volatile solids (VS) were measured directly on the samples in triplicate. Water extraction was performed for the measurement of pH, water-soluble carbohydrates (WSC), volatile fatty acids (VFA) and other metabolites. 30 gFM of sample were steeped in 150 mL of deionized water for 18 h at 4°C in sealed plastic pots in triplicate, as suggested by Porter & Murray (2001). Each liquid extract was used separately for pH and soluble compounds analysis. pH was measured directly after extraction on the mixture. Then, the liquid phase was separated by centrifugation (18750 g, 20 min, 4°C) and frozen in air-tightly closed tubes for further WSC, VFA and metabolites analysis.

2.4 Physicochemical analysis

TS were measured in triplicate by drying 30 g of sample at 105°C for 24 h. Volatile Solids (VS) were then measured by calcination of the dry residue (550°C, 3 h). Pretreated substrates can contain

volatile fatty acids (VFA), lactic acid (LA) and some alcohols that may evaporate during oven drying, causing underestimation of TS and VS. For this reason, the TS content value measured by oven drying was corrected using the equation proposed by Porter & Murray (2001) (Eq. (2)):

$$TS_C = TS_M + 0.375\ LA + 0.892\ VFA + 0.975\ Alcohols \qquad (2)$$

where TS_C = corrected TS; TS_M = measured TS; LA = lactic acid concentration; VFA = total VFA concentration; Alcohols = total alcohols concentration. All concentrations are in $g.gFM^{-1}$.

pH was measured in triplicate directly on the extraction mixture using a WTW® SenTix® 41 probe on a WTW® inoLab® pH7110. WSC and concentration of metabolites (i.e., lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (EtOH)) were measured from the centrifuged liquid phase after filtering (0,2 µm nylon filter) by High Performance Liquid Chromatography on Aminex 4PX-87H column (Bio-Rad) at 45°C. Sulfuric acid (0,005 M; 0,3 $mL.min^{-1}$) was used as mobile phase. WSC content was calculated as the sum of glucose, fructose, xylose and arabinose. Ammonia concentration was measured on liquid extract by titration with boric acid using a Gerhardt® Vapodest 50s® carousel. The gas composition was analyzed by gas chromatography (Perkin Elmer Clarus® 580). The volume of the gas sample was 200 µL and argon was used as carrier gas (350 kPa at 34 $mL\ min^{-1}$). After injection, CO_2 was separated from other gas by a capillary R-Q-bond column (30 m x 0.32 mm). H_2, O_2, N_2 and CH_4 were separated on a Rt-Molsieve 5 Å capillary column (30 m x 0.32 mm). Injector and thermal conductivity temperatures were set at 250 °C and 150 °C, respectively.

Fiber distribution was analyzed in triplicate using the Van Soest and Wine method (1967) from freeze-dried samples crushed to 1mm theoretical particle size. Water extract (W.EX), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and calcination

residue (CAL) content were determined. Water-soluble compounds (W.SOLU), neutral detergent soluble compounds (SOLU), hemicellulose (HEMI), cellulose (CELL) and lignin content (LIGN) were calculated as follow : W.SOLU = 1 – W.EX; SOLU = WEX – NDF; HEMI = NDF – ADF; CELL = ADF – ADL; LIGN = ADL – CAL. One-way analysis of variance (ANOVA) was used to test the difference in mean value between the different fiber fractions.

The total carbon (TC) and total nitrogen (TN) were determined via an elemental analyzer (FlashSmart®, Thermo Fisher Scientific®) on finely grounded freeze dried samples. TC and TN analysis were not replicated.

2.5 Biochemical Methane Potential Test

BMP was measured in batch assay. Samples were digested in 550 mL flasks with a working volume of 400 mL. Each flask is filled with 5 $gTS.L^{-1}$ of substrate, 5 gVS of anaerobic inoculum (UASB granular sludge), $NaHCO_3$ buffer ($50g.L^{-1}$), and macro- and microelement solutions as described by Monlau (Monlau et al., 2012). Before being closed, the flasks were flushed with N_2 to obtain anaerobic conditions. Triplicate bottles were incubated at 35°C. Controls containing only inoculum, buffer and macro- and microelement solutions were prepared in order to subtract the endogenous methane production of the inoculum from the one due to the samples digestion. The methane production was measured using an automatic batch test system (AMPTS® II, Bioprocess Control®, Sweden). Methane potentials are expressed as the volume of methane produced per amount of VS added for the BMP test, or per initial amount of VS estimated after taking into account the mass losses. BMP expressed in $NmLCH_4.gVS_{added}$ is useful to estimate substrate degradability after storage, while BMP expressed in $NmLCH_4.gVS_{initial}$.is mandatory to evaluate the

global balance of the alkaline pretreatment process. One-way analysis of variance (ANOVA) was used to test the difference in mean value between BMP of fresh and stored samples.

2.6 Theoretical gas production calculation

In addition to the measured volume by pressure difference and gas chromatography, the cumulated gas production at 180 days of CO_2 and H_2 was estimated from the associated metabolites. Since most of the microbial fermentations are accompanied by CO_2 and H_2 production, their respective stoichiometric reactions were used in the following equations, adapted from Hillion et al. (2018):

$$\text{Cumulated } CO_2 = \text{Acetic acid} + \text{Ethanol} + \text{Propionic acid} + 2\,\text{Butyric acid} + \text{Isobutyric acid} + \text{Valeric acid} + \text{Isovaleric acid} + \text{Caproic acid} \quad (3)$$

$$\text{Cumulated } H_2 = 2 \times [\text{Acetic acid} + \text{Butyric acid} + \text{Isobutyric acid} + \text{Valeric acid} + \text{Isovaleric acid}] \quad (4)$$

where cumulated CO_2 and H_2 are expressed in $mmol.kgVS_{init}^{-1}$ and all metabolites concentrations are expressed in $mmol.kgVS_{add}^{-1}$.

3 Results and Discussion

3.1 Raw material characterization

The main characteristics of the oat and rye fresh samples are summarized in Table 1. Both crops show a relatively low TS content when compared to other forage crops for silage making, whose optimal TS at harvest is around 30% (Teixeira Franco et al., 2016). These values are representative of what is expected for cover crops. Molinuevo-Salces et al. (2013) reported an average TS value of 17 ± 5 % and a variation from 10 to 29%TS for 20 different experimental cover crop assays. Cover crops usually have a low TS at harvest since they are cut before the optimal growth stage. However, since no water was added for the alkaline storage process, the TS value is high when compared to other alkaline pretreatment conditions in the literature, where TS is usually less than or equal to 10% (Khor et al., 2015; Monlau et al., 2013a, 2012; Thomas et al., 2018).

Both crops had a low nitrogen content, with C/N ratios of 59 and 38 for oat and rye, respectively. Cereals are reported to have a lower nitrogen content than legumes or grasses in the literature (Kaiser and Piltz, 2004).

Water-soluble carbohydrates (WSC) were mostly composed of fructose and glucose, which are the principal primary soluble sugars found in temperate forage grass (Downing et al., 2008). Initial concentrations in WSC of 131 $g.kgVS^{-1}$ and 66 $g.kg_{VS}^{-1}$ were measured for oat and rye, respectively. These are considered as medium (oat) to low (rye) when compared to other crops forages in temperate climate, whose WSC content varies from 50 to more than 300 $g.kg_{VS}^{-1}$ (Kaiser and Piltz, 2004). However, if a high WSC amount is essential to ensure a good quality of lactic fermentation in silage making, these soluble sugars can be used as easily accessible and biodegradable substrates for undesired fermentations in the present process. Fermentative metabolites such as

242 VFA, lactic acid or ethanol were not detected in the raw samples, indicating the absence of
243 fermentative activity during harvesting and storage prior to the start-up of the experiments.

244 Both crops present similar composition in fiber constituents in the range of commonly
245 encountered values in cover crops, with relatively high hemicellulose content (Molinuevo-Salces
246 et al., 2013)

247 3.2 Evolution of the chemical characteristics

248 The parameters allowing to follow the possible microbial activity and fermentative pathways
249 involved during storage were monitored all along the experiments. Table 2 presents the variation
250 of pH and ammonia concentration over storage period. Figure 1 shows the profile of the main
251 analyzed components at different studied storage times and the cumulated gas production. Figure
252 2 shows the distribution of the energy potential of the main analyzed compounds (WSC,
253 metabolites and gases) expressed in COD equivalent. Unexpectedly, the pH dropped very quickly
254 after closing the flasks and reached the neutrality in less than one week. At the same time,
255 increasing concentrations of organic acids were recorded. The loss of the alkaline conditions was
256 coupled to a quick WSC fermentation to VFA, lactic acid, CO_2 and H_2 (Figures 1 et 2). From the very
257 first days of the storage, different metabolic pathways occurred one after the other and were
258 associated with the accumulation and/or consumption of different kinds of metabolites (Figure 1).
259 Depending on the type of crop, even if some differences in the profile of metabolite
260 concentrations were observed, four main phases can be identified.

261 During the first two days, a fast and strong accumulation of acetic acid was measured, up to
262 concentration as high as 20.7 $g.kgVS_{add}^{-1}$ and 25.8 $g.kgVS_{add}^{-1}$ for oat and rye, respectively.
263 Interestingly, this production of acetic acid was not accompanied by any significant gas

264 production, and occurred at a high pH (between 12 and around 10). If this pH range is not
265 favorable to most of fermentative bacteria, examples of the literature reported high acetic acid
266 fermentation rates at elevated pH of 10 in mesophilic conditions (Dai et al., 2018; Ma et al., 2017)
267 and even 11 at 22°C (Yuan et al., 2006). It was shown that most bacteria could not survive to the
268 alkaline environment (Ma et al., 2017) and the cited works used inoculum from waste water
269 treatment plants or anaerobic reactors with a high initial microbial diversity, which was not the
270 case in the present work. A well-known abiotic chemical reaction, the deacetylation of
271 hemicellulose, could thus be implicated in alkaline conditions. Alkaline deacetylation of
272 lignocellulosic biomasses such as rice straw (Castro et al., 2017) and corn stover (Chen et al., 2014)
273 using a dilute NaOH pretreatment resulted in the release of up to 24 $g.kgVS^{-1}$ acetic acid by
274 solubilizing the acetyl groups from xylans of hemicellulose. This deacetylation reaction was carried
275 out at high temperature (50 to 80°C), with medium to high reagent concentration (20 to 80$g.kg_{TS}^{-1}$
276 NaOH), with short reaction time of less than two hours, making obvious the absence of
277 microorganism action in the process. The acetic acid release is strongly associated to the fast pH
278 drop during the first two days of the storage.

279 From day 2 to day 7, whatever the substrate, different soluble fermentative metabolites
280 accumulated, and in particular lactic and propionic acids (oat and rye), acetic acid (oat) and
281 ethanol (rye). The evidence of fermentative activities is also confirmed by the degradation of
282 glucose and fructose with a major decrease of their initial concentration. An increase in CO_2 and H_2
283 production rates was observed. The nature of the consumed sugars and produced metabolites,
284 including gases, indicate that heterolactic fermentation occurred, following one or the other of the
285 reactions below (Eq. (5) and (6)) (Hillion et al., 2018):

286 $$\text{Glucose/Fructose} + H_2O \rightarrow \text{Lactic acid} + \text{Acetic acid} + CO_2 + H_2O \quad (5)$$

287 $$\text{Glucose} \rightarrow \text{Lactic acid} + \text{Ethanol} + CO_2 \quad (6)$$

288 Heterolactic fermentations are commonly met in silage fermentation. However, the optimal pH
289 range for the growth of the involved lactic acid bacteria is in the range of 5 to 6, and their growth
290 rate is often reduced if the pH of the crop to be ensiled is equal to or greater than 7 (Driehuis et
291 al., 2003) even if some lactic fermentations were reported with a starting pH of 9.5 using selected
292 *Lactobacillus* strains as inoculum. This may evidence the existence of heterogeneous zones in the
293 substrate with a lower pH than the measured value, allowing the development of lactic acid
294 bacteria. The pH heterogeneity was probably due to the application of CaO as a dry powder
295 and/or the acetic acid release during the deacetylation phase.

296 From the second week of storage until day 60 (oat) or 180 (rye), acetic acid concentrations were
297 increasing, while certain metabolites accumulated after one week of storage were completely
298 (lactic acid, ethanol) or partially (acetic acid, propionic acid) converted into butyric acid. During
299 this period, residual glucose and fructose were completely consumed. High gas production was
300 observed during this phase, and the ratio between CO_2 and H_2 production flow rates remained
301 stable at $CO_2/H_2 = 2.5$. Butyric fermentation is due to bacteria of the *Clostridium* genus, which
302 commonly grow in insufficiently acidified silages. They grow on soluble carbohydrates (Eq. (7),
303 (Hillion et al., 2018)) and organic acids by a fermentation pathway that can be considered as the
304 sum of Eq. (8) and (9) (Driehuis et al., 2003).

305 $$\text{Glucose} \rightarrow \text{Butyric acid} + 2\,CO_2 + 2\,H_2 \quad (7)$$

306 $$\text{Lactic acid} \rightarrow \text{Acetic acid} + CO_2 + 2H_2 \quad (8)$$

307 $$\text{Lactic acid} + \text{Acetic acid} \rightarrow \text{Butyric acid} + CO_2 \quad (9)$$

In storage processes for anaerobic digestion, clostridial fermentations are considered as undesired, since they lead to the production of H_2 that escapes from the silo and induces losses of methane potential (Kreuger et al., 2011; Teixeira Franco et al., 2016).

In the present case, the total consumption of the previously produced lactic acid indicates that, at least, clostridial fermentations of lactic acid occurred. However, regarding the balance between degraded COD from initial WSC and cumulated metabolites (Figure 2), the observed sugar consumption could not explain alone the accumulation level of primary metabolites (LA, AA, PA, EtOH) and their further conversion into BA. This indicates that more complex carbohydrates were constantly degraded and released soluble sugars. These released WSC, mostly xylose, glucose and arabinose from the hemicellulose, may have been continuously converted into lactic acid (Eq. (5) or (10) (Hillion et al., 2018)) and then into butyric acid (Eq. (8) and (9)), or directly degraded into butyric acid following Eq. (7).

$$\text{Arabinose/Xylose} \rightarrow \text{Lactic acid} + \text{Acetic acid} \qquad (10)$$

The clostridial activity may also be evidenced by the fermentation of amino acids and amines into ammonia (Hillion et al., 2018). Table 2 shows a constant increase in ammonia concentration in rye, reaching 22% of initial TN after 180 days of storage. Surprisingly, a much lower ammonia accumulation was observed in oat, despite an important butyric fermentation. This could be explained by the fact that butyric fermentation started earlier in oat, with BA apparition before 7 days of storage, when pH was still lowering from 10.5 to 6.6. In these conditions of high pH, a large fraction of the ammonia may have undergone a faster volatilization in the form of NH_3. Total Nitrogen analysis could help to conclude on this hypothesis, but no such measurements were performed on stored samples in the present work.

During the last phase of the process, the microbial activity was considerably reduced, except an acetic acid production that continued following a slow kinetic. Oat samples reached this relative steady state 3 times faster than rye samples after only 60 days and the final concentration in BA was twice higher, what may be explained by the higher initial amount of WSC in oat. Interestingly, this state of relative stability began after BA reached a threshold value of 100g.gVS_{add}^{-1}, after what no further BA production was observed. BA may therefore have an inhibitory effect. However, the fact that rye samples also seem to reach a similar stable state at the end of the experiment from day 108, even with a 2.7 times lower BA amount, indicates that the decrease of the microbial activity is more probably due to the depletion of the accessible fermentation substrates.

3.3 Process impact on Fiber distribution, VS and BMP preservation.

The pretreatment effect on the fiber distribution of the crops was studied, and the results are presented in Figure 3. In Oat samples, the three most accessible fractions significantly varied, from 39.6 to 31.6%TS_{init} (W.SOLU), 1.1 to 4.3%TS_{init} (SOLU) and 26.3 to 11.8%TS_{init} (HEMI) fractions significantly varied ($p < 0.05$). Rye samples undergone a lower transformation, with significant changes ($p < 0.05$) for SOLU (27.0 to 25.3%TS_{init}) and HEMI (29.4 to 17.7%TS_{init}) fractions only. In both cases, no solubilization of the cellulose and lignin fractions were observed. The hemicellulose was greatly reduced, and easily accessible carbohydrates were released, mostly xylose, glucose and arabinose. This WSC release explains the difference in the balance between the consumed COD of the initial WSC and the accumulated COD of the metabolites (section 3.2 and Figure 2). Nonetheless, since no effect on cellulose and lignin were observed, the pretreatment action of CaO was seemingly limited, and the long-term effect of the pretreatment on lignin wasn't obtained.

352 Figure 4 shows the dynamics of mass losses for both crops over the whole storage period. The gas
353 production related to microbial fermentations was responsible for significant VS losses of 18.1%
354 and 9.0% respectively for oat and rye during the experiment. The measured values were higher
355 (oat) or comparable (rye) to the ones that are commonly observed in ensiling, where 5 to 12% TS
356 losses occur during the fermentation of properly managed silages (Kaiser and Piltz, 2004).

357 The VS losses were mostly due to CO_2 production. Mass losses and cumulated CO_2 production
358 followed the same behavior (Figures 1 et 3). Considering mass balance during alkaline
359 pretreatments, mass losses computed on the base of the mass of gas release were 123 and 40
360 $g.kgVS_{init}^{-1}$ for oat and rye respectively and were significantly lower than direct mass measurement
361 of the jars (181 and 90 $g.kgVS_{init}^{-1}$) respectively. This may evidence the presence of gas leaches on
362 the experimental setup over the 6-month period, as direct weighting of jar is a more reliable
363 method. Considering the theoretical gas production based on the accumulated metabolites
364 calculated as presented in section 2.6, cumulated CO_2 and H_2 should theoretically be of 3572 and
365 4341 $mmol.kgVS_{init}^{-1}$ for oat, 2207 and 3158.4 $mmol.kgVS_{init}^{-1}$ for rye.

366 The significant difference between these values and the measured ones presented in Figure 1,
367 particularly considering the CO_2/H_2 ratio, may also indicates that in-situ gas consumption like
368 homoacetogenesis took place, converting H_2 and CO_2 into acetic acid (Eq. (11), (Hillion et al.,
369 2018)).

370 $$4\ H_2 + 2\ CO_2 \rightarrow \text{Acetic acid} + 2\ H_2O \qquad (11)$$

371 This phenomenon occurs in dark fermentation, where in-situ H_2 consumption by homoacetogenic
372 bacteria is reported, particularly at pH close to 7 (Saady, 2013). Consequently, the effective energy
373 losses due to H_2 production were low.

374 BMP tests were carried out on the raw substrates and on substrates stored for 7, 60 and 180 days.
375 Figure 5 summarizes the methane potential expressed in $NmLCH_4.gVS_{add}^{-1}$ and $NmLCH_4.gVS_{init}^{-1}$, as
376 detailed in section 2.5. The raw oat and rye samples BMP were respectively 294 ± 3 and 348 ± 11
377 $NmLCH_4.gVS_{init}^{-1}$. These values are comparable to other BMP of cereal cover crops that commonly
378 vary from 200 to 400 $NmLCH_4.gVS^{-1}$ in the literature (Molinuevo-Salces et al., 2013).

379 Whatever the cover crop, the degradability of the substrate increased during the alkaline
380 pretreatment storage by 29% (oat) and 8.5% (rye) after 180 days, suggesting at first glance a
381 significant ($p = 0.015$ and 0.056, respectively) action of the pretreatment on the lignocellulosic
382 biomass. However, after taking into account the mass losses during the storage, the methane
383 potential only varied by +6% and -1% for oat and rye, respectively, which indicates no significant
384 difference from the BMP of the fresh substrates ($p = 0.355$ and 0.676, respectively). During the
385 storage period, the production of H_2 can be the cause of an energy loss, and thus of the reduction
386 of the methane potential. This loss was estimated by calculating the COD of H_2 produced (Figure 2)
387 and it represented 5.4 and 2.2 $NmLCH_4.gVS^{-1}$ for oat and rye, respectively 1.8 and 0.6% of initial
388 BMP. This theoretical value is very low compared to that of the BMP of the fresh substrate, which
389 may explain why no loss of methane potential was experimentally observed during storage. In
390 well-preserved silages, slightly higher energy losses from 2 to 4% happen during fermentation
391 (Kaiser and Piltz, 2004). Consequently, during the alkaline storage, because of the loss of mass of
392 the substrate in the form of CO_2 and the conversion of the initial soluble carbohydrates (373 NmL
393 $CH_4.g^{-1}$) into more energetic compounds such as butyric acid that were released (636 $NmLCH_4.g^{-1}$),
394 the methane potential was concentrated in the remaining dry matter. Figure 5 presents the
395 estimated BMP of the present metabolites in the substrates, in $NmLCH_4.gVS_{add}^{-1}$. The increase of
396 the BMP of the metabolites was strongly linked to the increase of the substrate degradability and

mass losses, showing the concentration of the BMP of the crop in the remaining biomass after storage.

Thus, negligible BMP losses occurred considering the whole process, and the initial methane potential of the harvest was efficiently preserved, despite microbial reactions considered as highly undesirable in crop storage processes such as butyric fermentation. By extension, this work brings interesting data for understanding the impact of ensiling on crops for biogas production. In fact, the substrate characteristics along with the microbial fermentation pathways that took place after the first days (pH close to neutrality and important butyric fermentation) is highly comparable to a silage fermentation considered as of a very poor quality. The quality of silage for animal feeding fulfills the following criteria: pH < 4.5, lactic acid > 30g.kgTS^{-1}, butyric acid < 10% of total VFA (Bureenok et al., 2016), while the present stored substrate is far from these characteristics, showing that ensiling for biogas production has very different purposes than for animal feed. This suggests that the ideal silage characteristics for anaerobic digestion are not well defined yet, and future researches may address this subject.

Considering the initial pretreatment objective of the process, it can be concluded that the alkaline conditions were not maintained long enough to obtain the expected long-term pretreatment and increase the BMP. However, even if a significant increase is not already reached, the elevated pH (pH 7) of the stored crop could lower the detrimental effect of organic acids on the concrete walls of the silo, which is a reported problem with acid silages (pH 4) (Koenig and Dehn, 2016). Research needs to be undertaken in order to reach the optimal conditions of operation by focusing on the initial characteristics like the alkaline reactive nature and load, global TS and the presence of available substrates for microbial fermentation, with the purpose of maintaining the alkaline conditions for a sufficient time in order to increase the pretreatment efficiency.

4 Conclusions

A succession of abiotic and biotic reactions induced a fast and sharp pH drop from 12 to 7, and thus compromised the long-term action of the alkaline agent. Despite undesirable fermentations and high mass losses, no significant effect on the methane potential was observed during the storage process. Alkaline storage, however, induced an increase in specific biodegradability which is associated with mass loss. This study clearly demonstrates that the methanogenic potential of the two selected cover crops was maintained for more than 6 months. An optimized process under stable alkaline conditions could therefore allow a better pretreatment efficiency.

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References

Atelge, M.R., Atabani, A.E., Banu, J.R., Krisa, D., Kaya, M., Eskicioglu, C., Kumar, G., Lee, C., Yildiz, Y., Unalan, S., Mohanasundaram, R., Duman, F., 2020. A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. Fuel 270. https://doi.org/10.1016/j.fuel.2020.117494

Büchi, L., Wendling, M., Amossé, C., Jeangros, B., Charles, R., 2020. Field Crops Research Cover crops to secure weed control strategies in a maize crop with reduced tillage. F. Crop. Res. 247, 107583. https://doi.org/10.1016/j.fcr.2019.107583

Bureenok, S., Sisaath, K., Yuangklang, C., Vasupen, K., Schonewille, J.T., 2016. Ensiling characteristics of silages of Stylo legume (Stylosanthes guianensis), Guinea grass (Panicum maximum) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages. Small Rumin. Res. 134, 84–89. https://doi.org/10.1016/j.smallrumres.2015.12.006

Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., Ferrer, I., 2016. Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application. Bioresour. Technol. 199, 386–397. https://doi.org/10.1016/J.BIORTECH.2015.09.007

Castro, R.C. de A., Fonseca, B.G., dos Santos, H.T.L., Ferreira, I.S., Mussatto, S.I., Roberto, I.C., 2017. Alkaline deacetylation as a strategy to improve sugars recovery and ethanol production from rice straw hemicellulose and cellulose. Ind. Crops Prod. 106, 65–73. https://doi.org/10.1016/j.indcrop.2016.08.053

Chen, X., Shekiro, J., Pschorn, T., Sabourin, M., Tao, L., Elander, R., Park, S., Jennings, E., Nelson, R., Trass, O., Flanegan, K., Wang, W., Himmel, M.E., Johnson, D., Tucker, M.P., 2014. A highly efficient dilute alkali deacetylation and mechanical (disc) refining process for the conversion of renewable biomass to lower cost sugars. Biotechnol. Biofuels 7. https://doi.org/10.1186/1754-6834-7-98

Dai, K., Zhang, F., Zhang, Y., Zeng, R.J., 2018. The chemostat metabolite spectra of alkaline mixed culture fermentation under mesophilic, thermophilic, and extreme-thermophilic conditions. Bioresour. Technol. 249, 322–327. https://doi.org/10.1016/j.biortech.2017.10.035

Digman, M.F., Shinners, K.J., Casler, M.D., Dien, B.S., Hatfield, R.D., Jung, H.-J.G., Muck, R.E., Weimer, P.J., 2010. Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. Bioresour. Technol. 101, 5305–5314. https://doi.org/10.1016/J.BIORTECH.2010.02.014

Downing, T.W., Buyserie, A., Gamroth, M., French, P., 2008. Effect of Water Soluble Carbohydrates on Fermentation Characteristics of Ensiled Perennial Ryegrass. Prof.

Anim. Sci. 24, 35–39. https://doi.org/10.15232/S1080-7446(15)30807-X

Driehuis, F., Elferink, S.O., Spoelstra, S.F., 2003. Microbiology of Ensiling. https://doi.org/10.2134/agronmonogr42.c2

Elferink, S.J.W.H.., Driehuis, F., Gottschal, J.C., Spoelstra, S.F., 1999. Silage fermentation processes and their manipulation. FAO Plant Prod. Prot. Pap. 17–30.

Goffart, A., 1877. Manuel de la culture et de l'ensilage de maïs et autres fourrages verts [Manual of the cultivation and siloing of maize and other green fodders]. Paris.

Hillion, M. Lou, Moscoviz, R., Trably, E., Leblanc, Y., Bernet, N., Torrijos, M., Escudié, R., 2018. Co-ensiling as a new technique for long-term storage of agro-industrial waste with low sugar content prior to anaerobic digestion. Waste Manag. 71, 147–155. https://doi.org/10.1016/j.wasman.2017.10.024

Hillion, M., 2017. Optimisation de la digestion anaérobie en voie sèche continue de résidus lignocellulosiques. PhD thesis, Montpellier Supagro.

Igos, E., Golkowska, K., Koster, D., Vervisch, B., Benetto, E., 2016. Using rye as cover crop for bioenergy production: An environmental and economic assessment. Biomass and Bioenergy 95, 116–123. https://doi.org/10.1016/j.biombioe.2016.09.023

Jian, J., Du, X., Reiter, M.S., Stewart, R.D., 2020. A meta-analysis of global cropland soil carbon changes due to cover cropping. Soil Biol. Biochem. 143, 107735. https://doi.org/10.1016/j.soilbio.2020.107735

Jiang, D., Ge, X., Zhang, Q., Zhou, X., Chen, Z., Keener, H., Li, Y., 2017. Comparison of sodium hydroxide and calcium hydroxide pretreatments of giant reed for enhanced enzymatic digestibility and methane production. Bioresour. Technol. 244, 1150–1157. https://doi.org/10.1016/J.BIORTECH.2017.08.067

Jury, C., Benetto, E., Koster, D., Schmitt, B., Welfring, J., 2010. Life Cycle Assessment of biogas production by monofermentation of energy crops and injection into the natural gas grid. Biomass and Bioenergy 34, 54–66. https://doi.org/10.1016/j.biombioe.2009.09.011

Kaiser, A.G., Piltz, J.W., 2004. Successful silage.

Khor, W.C., Rabaey, K., Vervaeren, H., 2015. Low temperature calcium hydroxide treatment enhances anaerobic methane production from (extruded) biomass. Bioresour. Technol. 176, 181–188. https://doi.org/10.1016/J.BIORTECH.2014.11.037

Koenig, A., Dehn, F., 2016. Biogenic acid attack on concretes in biogas plants. Biosyst. Eng. 147, 226–237. https://doi.org/10.1016/j.biosystemseng.2016.03.007

Kreuger, E., Nges, I., Björnsson, L., 2011. Ensiling of crops for biogas production: Effects on methane yield and total solids determination. Biotechnol. Biofuels 4, 1–8.

https://doi.org/10.1186/1754-6834-4-44

Ma, H., Liu, He, Zhang, L., Yang, M., Fu, B., Liu, Hongbo, 2017. Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol. Biofuels. https://doi.org/10.1186/s13068-017-0821-1

McNulty, P.B., Kennedy, S., 1982. Density Measurements of Grass by Toluene Displacement and Air Comparison Pycnometry. Irish J. Agric. Reserach 21, 75–83.

Molinuevo-Salces, B., Larsen, S.U., Ahring, B.K., Uellendahl, H., 2013. Biogas production from catch crops: Evaluation of biomass yield and methane potential of catch crops in organic crop rotations. Biomass and Bioenergy 59, 285–292. https://doi.org/10.1016/j.biombioe.2013.10.008

Monlau, F., Aemig, Q., Barakat, A., Steyer, J.P., Carrère, H., 2013a. Application of optimized alkaline pretreatment for enhancing the anaerobic digestion of different sunflower stalks varieties. Environ. Technol. (United Kingdom) 34, 2155–2162. https://doi.org/10.1080/09593330.2013.808247

Monlau, F., Barakat, A., Steyer, J.P., Carrere, H., 2012. Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. Bioresour. Technol. 120, 241–247. https://doi.org/10.1016/j.biortech.2012.06.040

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P., Carrère, H., 2013b. Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment. Crit. Rev. Environ. Sci. Technol. 43, 260–322. https://doi.org/10.1080/10643389.2011.604258

Porter, M.G., Murray, R.S., 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. Grass Forage Sci. 56, 405–11. https://doi.org/10.1046/j.1365-2494.2001.00292.x

Saady, N.M.C., 2013. Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: Unresolved challenge. Int. J. Hydrogen Energy 38, 13172–13191. https://doi.org/10.1016/j.ijhydene.2013.07.122

Sambusiti, C., Ficara, E., Malpei, F., Steyer, J.P., Carrère, H., 2012. Influence of alkaline pre-treatment conditions on structural features and methane production from ensiled sorghum forage. Chem. Eng. J. 211–212, 488–492. https://doi.org/10.1016/j.cej.2012.09.103

Sapkota, T.B., Askegaard, M., Lægdsmand, M., Olesen, J.E., 2012. Effects of catch crop type and root depth on nitrogen leaching and yield of spring barley. F. Crop. Res. 125,

129–138. https://doi.org/10.1016/j.fcr.2011.09.009

Teixeira Franco, R., Buffière, P., Bayard, R., 2016. Ensiling for biogas production: Critical parameters. A review. Biomass and Bioenergy 94, 94–104. https://doi.org/10.1016/j.biombioe.2016.08.014

Thomas, H., Seira, J., Escudié, R., Carrère, H., 2018. Lime Pretreatment of Miscanthus: Impact on BMP and Batch Dry Co-Digestion with Cattle Manure. Molecules 23, 1608. https://doi.org/10.3390/molecules23071608

Van Soest, P.J., Wine, R.H., 1967. Use of Detergents in the Analysis of Fibrous Feeds . IV . Determination of Plant Cell-Wall Constituents. J. Assoc. Off. Anal. Chem. 50, 50–55.

Weinberg, Z.., Ashbell, G., 2003. Engineering aspects of ensiling. Biochem. Eng. J. 13, 181–188. https://doi.org/10.1016/S1369-703X(02)00130-4

Yuan, H., Chen, Y., Zhang, H., Jiang, S., Zhou, Q., Gu, G., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40, 2025–2029. https://doi.org/10.1021/es052252b

559

560 Table and Figure Captions

561 Table 1: Physicochemical characteristics of the catch crops

562 Table 2: pH and NH_4-N variation during storage.

563 Figure 1: WSC, gas and metabolites variation during storage. Lactic acid (LA), Acetic acid (AA),
564 Propionic Acid (PA), Butyric acid (BA) and Ethanol (ETOH) are expressed in mmol.kgVS_{add}^{-1}. CO_2 and
565 H_2 are expressed in cumulated mmol/kgVS_{init}^{-1}. The error bars indicate standard deviation.

566 Figure 2: Variation of the COD repartition between WSC, soluble metabolites and gases. The error
567 bars indicate standard deviation.

568 Figure 3: Alkaline storage effect on fiber distribution. The error bars indicate standard deviation.

569 Figure 4: Dynamics of mass losses. The error bars indicate standard deviation.

570 Figure 5: Impact of storage duration on methane potential. BMP ($NmLCH_4.gVS_{added}^{-1}$) and BMP
571 ($NmLCH_4.gVS_{initial}^{-1}$) represent pretreated crop biodegradability and methane potential reported to
572 the VS amount before pretreatment, respectively. $BMP_{metabolites}$ represent the contribution of
573 soluble metabolites in BMP ($NmLCH_4.gVS_{initial}^{-1}$). The error bars indicate standard deviation.

Oat

Rye

Amount (mmol.kgVS$^{-1}_{add}$)

2000

1000

0

1200

800

400

0

0

50

100

150

Time (d)

LA

AA

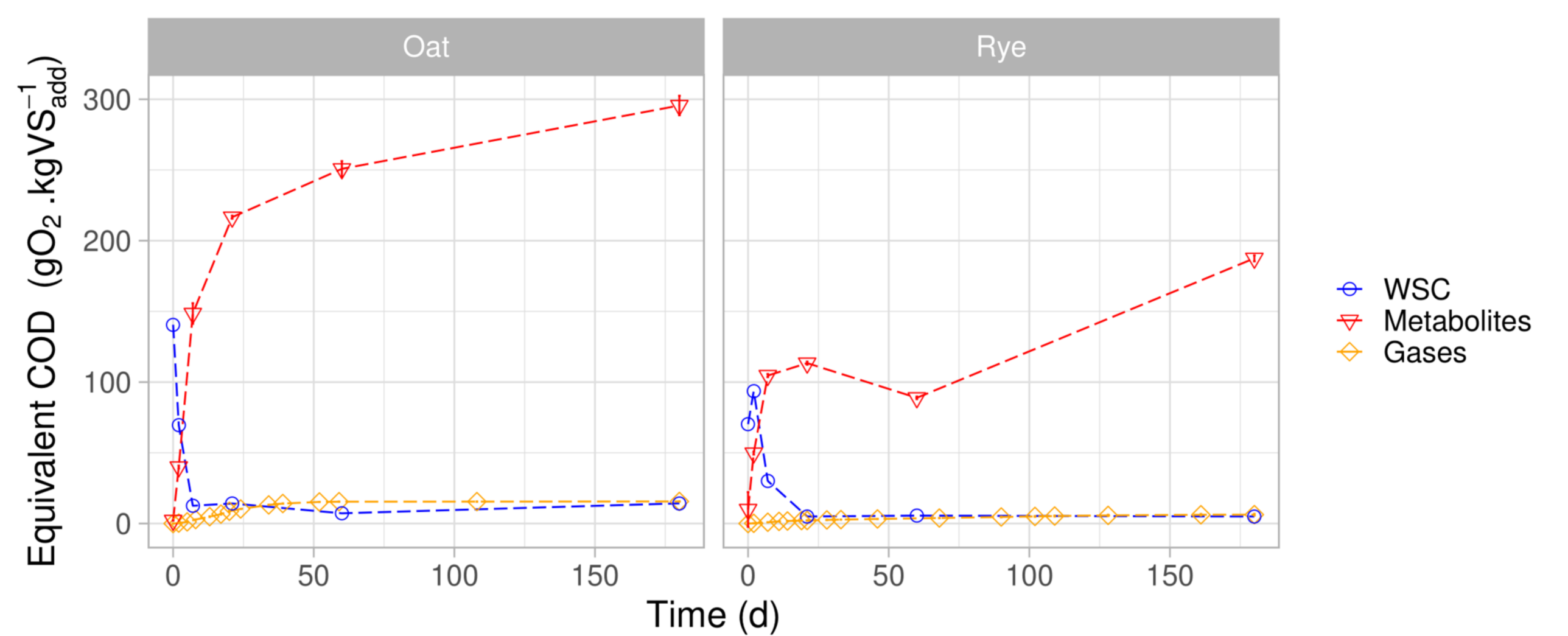
PA

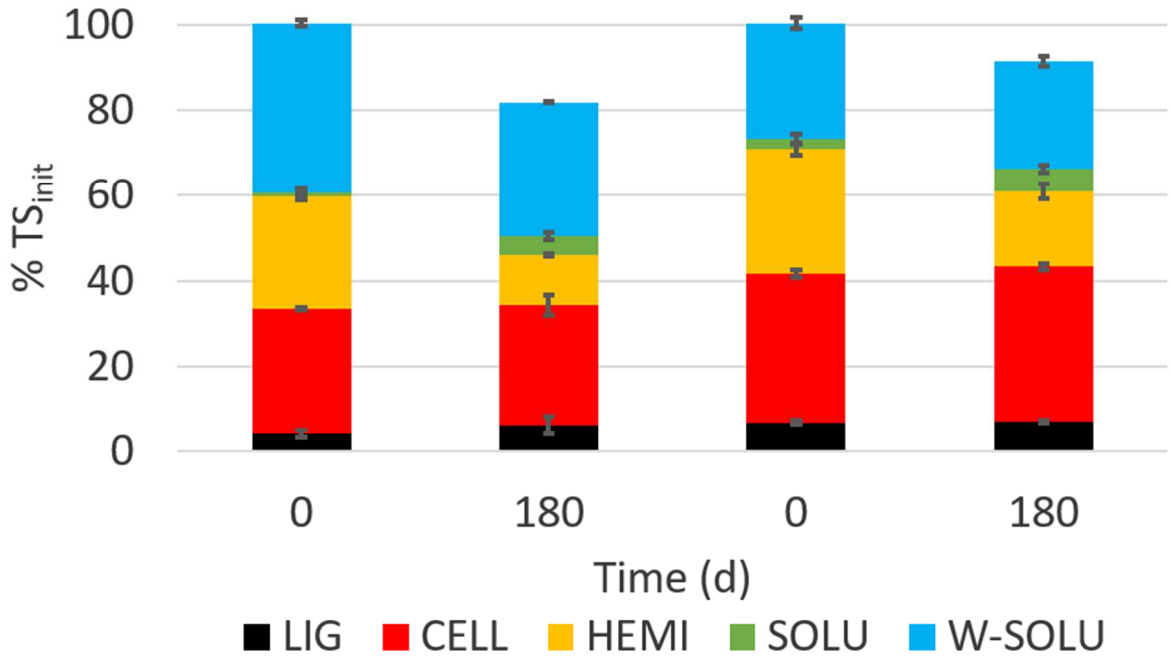
BA

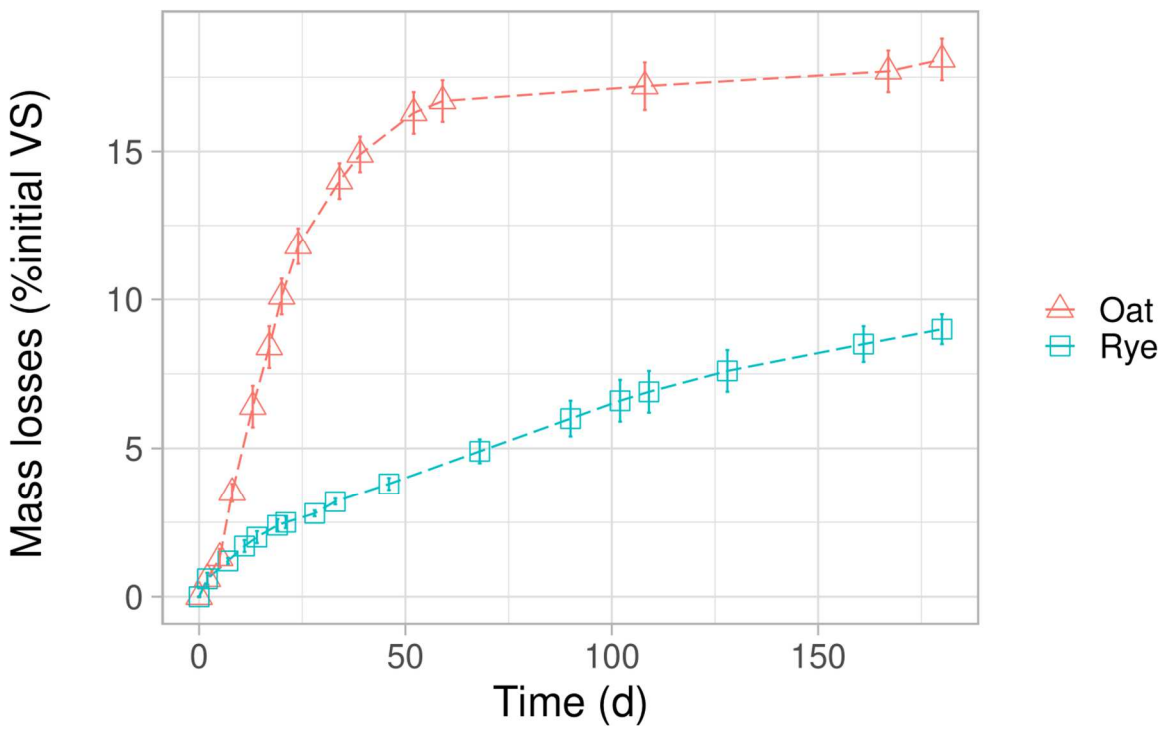
ETOH

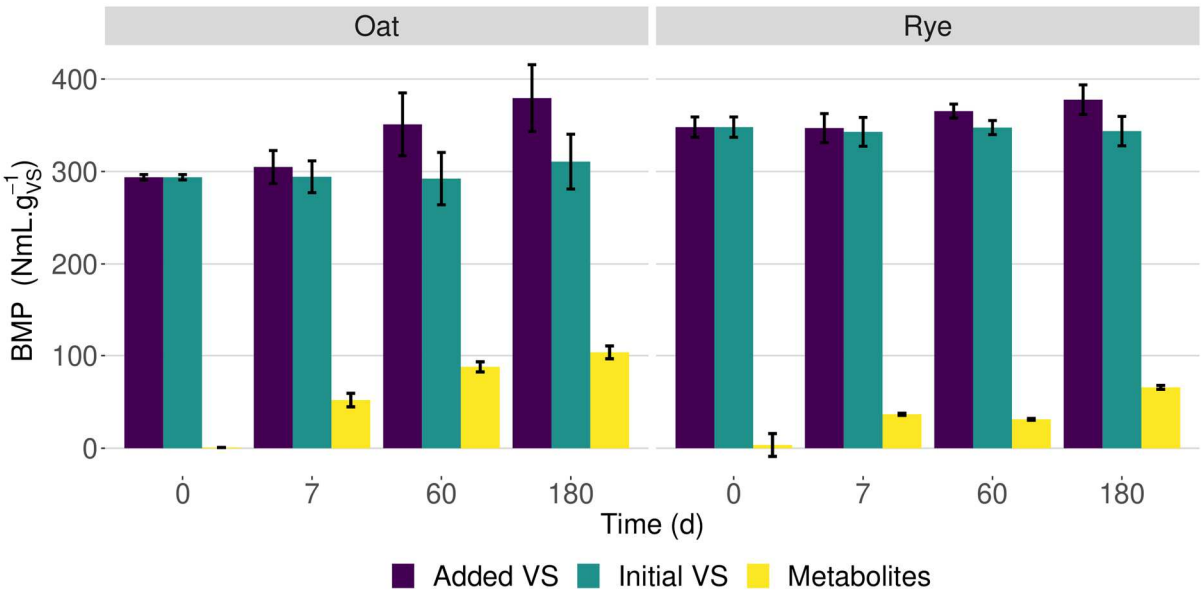
CO2

H2


Oat
Rye
Equivalent COD (gO2 .kgVS−1add)
300
200
100
0
0
50
100
150
Time (d)
WSC
Metabolites
Gases

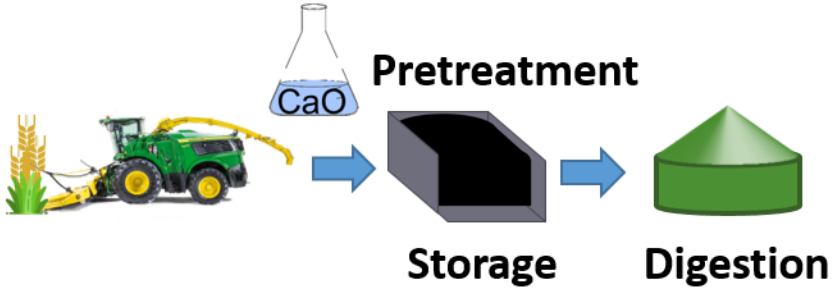

100
80
60
40
20
0
% TS$_{init}$
0
180
0
180
Time (d)
LIG
CELL
HEMI
SOLU
W-SOLU

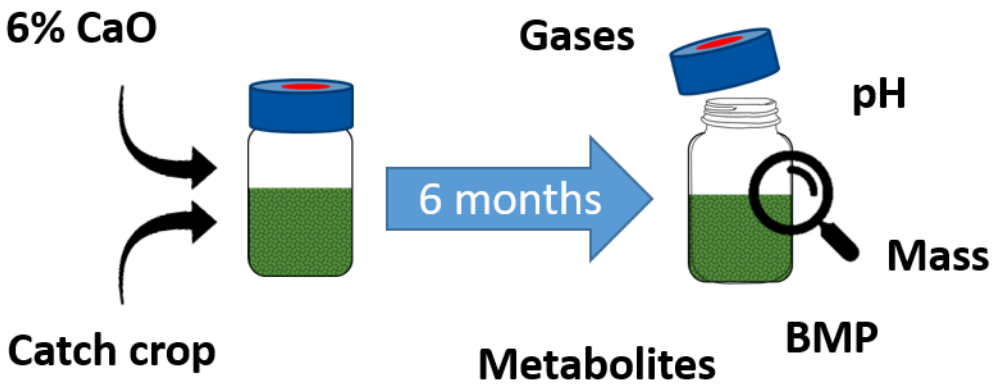

Mass losses (%initial VS)
15
10
5
0
0
50
100
150
Time (d)
Oat
Rye

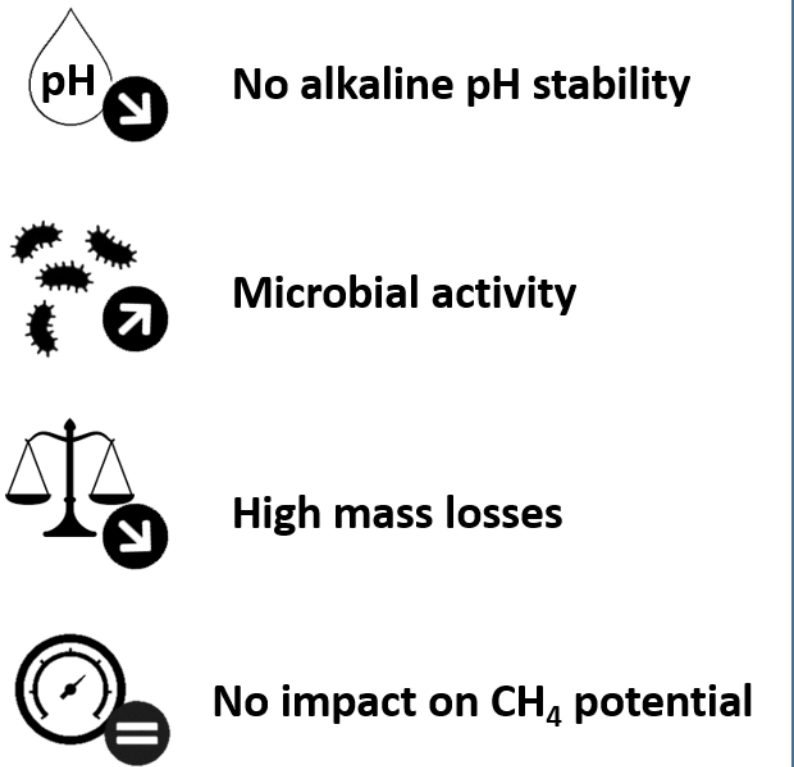

Oat
Rye
400
300
200
100
0
BMP (NmL.g$^{-1}_{VS}$)
0
7
60
180
Time (d)
Added VS
Initial VS
Metabolites

	Oat	*Rye*
pH	6.0 ± 0.0	6.0 ± 0.0
TS (%FM)	21.8 ± 0.01	24.9 ± 0.03
VS (%FM)	20.8 ± 0.01	23.7 ± 0.03
TN (%VS)	0.7	1.1
TC (%VS)	41.4	42.1
VFA ($g.kg_{VS}^{-1}$)	< d. l.	< d. l.
Fructose ($g.kg_{VS}^{-1}$)	76.6 ± 0.8	34.0 ± 0.4
Glucose ($g.kg_{VS}^{-1}$)	54.7 ± 0.7	31.4 ± 0.1
WSC ($g.kg_{VS}^{-1}$)	131 ± 1	66 ± 0
EtOH ($g.kg_{VS}^{-1}$)	< d. l.	< d. l.
W.SOLU (%TS)	39.6 ± 0.7	27 ± 1.4
SOLU (%TS)	1.1 ± 0.8	2.4 ± 1.2
HEMI (%TS)	26.3 ± 0.7	29.4 ± 1.5
CELL (%TS)	29.3 ± 0.2	34.9 ± 0.9
LIG (%TS)	4.1 ± 0.8	6.8 ± 0.5

	Time (d)	0	2	7	21	60	180
Oat	*pH*	11.9 ± 0.1	10.5 ± 0.0	6.6 ± 0.1	7.1 ± 0.1	7.5 ± 0.0	6.9 ± 0.0
	NH4-N ($g.kgVS_{add}^{-1}$)	0.2 ± 0	0.8 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.2
Rye	*pH*	12 ± 0.1	9.8 ± 0.0	7.2 ± 0.0	7.2 ± 0.1	7.9 ± 0.1	7.7 ± 0.1
	NH4-N ($g.kgVS_{add}^{-1}$)	0.3 ± 0	0.6 ± 0.1	1.1 ± 0	1.7 ± 0.1	2.3 ± 0	2.7 ± 0.1


CaO
Pretreatment
Storage
Digestion


6% CaO
Catch crop
6 months
Gases
pH
Mass
BMP
Metabolites


pH
No alkaline pH stability
Microbial activity
High mass losses
No impact on CH_4 potential