Methane potentials and organic matter characterization of wood fibres from pulp and paper mills: The influence of raw material, pulping process and bleaching technique

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ARTICLE INFO

Keywords:
Fibres
Softwood
Hardwood
Pulping
Bleaching
Anaerobic digestion

ABSTRACT

During the process of pulp- and papermaking, large volumes of fibre-rich primary sludge are generated. Anaerobic digestion of primary sludge offers a substantial potential for methane production as an alternative approach to the inefficient energy recoveries by commonly used incineration techniques. Therefore, biochemical methane potentials were determined at mesophilic conditions for 20 types of fibres processed by a variety of pulping and bleaching techniques and from different raw materials. This included fibres from kraft, sulphite, semi-chemical, chemical thermo-mechanical (CTMP) and thermo-mechanical pulping plants and milled raw wood. The pulping technique was clearly important for the methane potential, with the highest potential achieved for kraft and sulphite fibres (390–400 Nml CH₄ g VS⁻¹) and 300 compared to 160 Nml CH₄ g VS⁻¹, respectively). Nuclear magnetic resonance characterization of the organic content demonstrated that the relative lignin content of the fibres was an important factor for methane production, and that an observed positive effect of bleaching on the methane potential of softwood CTMP fibres was likely related to a higher degree of deacetylation and improved accessibility of the hemicellulose. In conclusion, fibres from kraft and sulphite pulping are promising substrates for methane production irrespective of raw material or bleaching, as well as fibres from CTMP pulping of hardwood.

1. Introduction

Large amounts of organic material are released during the process of pulp- and papermaking, resulting in high wastewater treatment costs for the industry [1]. The first step in wastewater treatment at pulp and paper mills is primary sedimentation/flotation, which generates large volumes of fibre sludge, often referred to as primary sludge (80–90 000 tonnes in Sweden in 2012 [2]). The primary sludge is, however, commonly dewatered and incinerated at low energy recovery due to the high moisture content [3]. Different production routes within the pulp and paper industry leading to accumulation of primary sludge is shown in Fig. 1, most of which may affect the possibility to produce biogas from this waste fraction. We have previously shown that there is substantial potential for methane production via anaerobic digestion (AD) of fibres in pulp and paper mill wastewater [4]. In addition to the production of a valuable energy carrier in the form of methane, AD of organic waste leads to a reduction of the waste volume and reduces the amount of sludge to be disposed of [5].

Abbreviations: AD, anaerobic digestion; BMP, biochemical methane potential; CPMAS, cross-polarization magic angle spinning; CTMP, chemical thermo-mechanical pulping; ECF, elemental chlorine-free; Hw, hardwood; HRT, hydraulic retention time; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; NSSC, neutral sulphite semi-chemical; Sw, softwood; TCF, total chlorine-free; TMP, thermo-mechanical pulping; TOC, total organic carbon; TS, total solids; VS, volatile solids.

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https://doi.org/10.1016/j.biombioe.2020.105824
Received 11 June 2020; Received in revised form 2 October 2020; Accepted 7 October 2020
Available online 27 October 2020
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Pulp and paper production is often carried out in campaigns, shifting the production between unbleached and bleached qualities of pulp while varying the raw materials used (Fig. 1). If such shifts lead to large variations in the methane potentials of the pulped fibres, the use of these fibres in a biogas production facility would lead to unwanted fluctuations in methane production, as well as variations in the composition of the digestate due to the accumulation of undigested fibres in the digesters. There are indications in the literature that the methane potential of pulped fibres may vary depending on the pulping process used, and in particular, demonstrating high methane potentials for fibres from kraft or sulphite pulping (Table 1). However, a systematic study on the influence of raw material and commonly used pulping and bleaching techniques on the methane potential of this type of fibre is lacking.

The aims of the present study were to (i) assess the methane potential of pulped fibres produced using common pulping or bleaching techniques for different wood materials, and (ii) elucidate the underlying mechanisms for any observed differences in methane potentials of the fibres. To address these aims, a systematic study of the methane production between unbleached and bleached qualities of pulp while varying the raw materials used (Fig. 1). If such shifts lead to large variations in the methane potentials of the fibres from mechanical, semi-chemical, kraft, and sulphite pulping techniques for different wood materials, and (ii) elucidate the underlying mechanisms for any observed differences in methane potentials of the fibres from mechanical, semi-chemical, kraft, and sulphite pulping and paper plants was undertaken. The structural characteristics of the fibre samples were studied using solid-state $^{13}$C cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy in relation to the methane potentials. This method is a well-established technique to characterize lignocellulosic materials by generating chemical fingerprints of major constituents of wood samples without the need for solvent extraction [10]. In addition, 2D solution-state $^{1}$H–$^{13}$C heteronuclear single quantum coherence (HSQC) NMR was also performed, as the superior resolution of this technique allows identification of the molecular structures in complex organic samples— not possible using 1D NMR techniques [11,12].

2. Material and methods

2.1. Fibre sampling and biochemical methane potential (BMP) tests

A summary of the different types of fibres investigated is presented in Table 2. When possible, both softwood fibres and hardwood fibres were sampled from the same pulping process. Fibre samples were collected from the wastewater after pulping and bleaching for the thermo-mechanical pulping (TMP) and the softwood chemical thermo-mechanical (CTMP) processes, whereas pulp was sampled directly from the product line for kraft, sulphite and neutral sulphite semi-chemical pulping (NSSC) processes. In addition, untreated wood chips from hardwood and softwood were sampled, dried for 1 h at 105 °C and milled to a particle size of <1 mm (raw fibres). The collection of all samples followed the sampling procedures implemented by mill personnel for regular process monitoring.

The methane potential was determined using biochemical methane potential (BMP) tests as described by Ekstrand et al. [4]. In short, the tests were performed using 320 ml glass bottles sealed with rubber stoppers and aluminium screw caps. 20 ml of inoculum (digestate from a municipal wastewater treatment plant, Linköping, Sweden) was added together with fibre sludge, nutrient salts and tap water up to a final volume of 100 ml. The organic loads in the batch bottles varied depending on the degradability of the substrates, ranging between 2.3–3.5 g volatile solids (VS) per litre for chemically pulped fibre samples, 4–5 g VS/L for CTMP and TMP-pulped fibres, and 6.7–8 g VS/L for raw fibres. This resulted in inoculum to substrate ratios of 1.4–2.0 for chemically pulped fibres, 0.9–1.4 for CTMP and TMP fibres, and 0.4–0.6 for raw fibres (see Appendix A for TS, VS and inoculum to substrate ratios for all samples). The ratio for CTMP, TMP and raw fibres is lower than 0.6.

![Fig. 1. Overview of the process steps involved in pulp- and papermaking that potentially affect the methane potential of the primary sludge. Mechanical pulping techniques include thermo-mechanical pulping (TMP) and chemical thermo-mechanical pulping (CTMFP), followed by bleaching with peroxide or sodium dithionite. Chemical pulping techniques include kraft and sulphite pulping, followed by elemental chlorine-free bleaching (ECF) or total chlorine-free bleaching (TCF). Neutral sulphite semi-chemical pulping (NSSC) combines a mild chemical pulping step with a mechanical refining step. Dashed lines denote the wastewater streams from the different process steps that are passed through primary clarification where residual fibres are collected as primary sludge.](image-url)
than for the chemically pulped fibres due to their lignocellulosic properties, and is in line with the recommendations for lignocellulosic materials outlined by Holliger et al. All tests were performed in triplicates and incubated in the dark at 37 °C. The gas production was determined by pressure measurements (Testo 312-2 Precision manometer, Testo Inc, USA), and its methane content was determined by gas chromatography with a flame ionizing detector (GC-FID). All measured gas volumes were normalized to 1 atm pressure and 273 K and are expressed as Normalized mL (Nml). After the completion of the BMP tests, methane potentials were evaluated as the amount of methane produced per gram VS of substrate added, minus control (NmL CH₄ g VS⁻¹). TS and VS were measured in duplicate according to the Swedish Standard method (SS 028113). The BMP test for each sample was ended when the cumulative gas production curve levelled off, generally after 40–60 days of incubation. However, for a few samples, the BMP test was ended before the maximum potential was achieved (CTMP softwood, TMP softwood and raw fibres), since the digestion time was too long (>96 days) to be reasonable in full-scale applications. Student’s t-tests (independent samples) were used to determine statistical significance (α < 0.5 and α < 0.01) between BMP samples.

Klason lignin was determined by applying acid hydrolysis of the fibre samples, followed by a gravimetric determination of the insoluble lignin (TAPPI T222). The remaining acid-soluble lignin was determined spectrophotometrically at 204 nm (TAPPI U1250). The composition of soluble compounds of the bleaching wastewater from the TMP mill was determined by mill personnel after solid-phase extraction (internal method, W35:97), followed by analyses of fatty- and resin acids, lignans, sterols, sterol esters, and triglycerides. Carbohydrates included arabino, xylose, mannose, galactose, and glucose and were determined according to an internal standard (C29:91) adapted from the method SCAN-CM71. Organic acids included butyric acid, propanoic acid, formic acid and acetic acid. Not all details of the analyses were shared by the mill, so these results are presented primarily as a support to the discussion of this study.

2.2. Kinetic modelling

To estimate the maximal rate of methane production (kmax) during the BMP tests of the fibre samples, a modified Gompertz equation was fitted to the experimental data of each sample. The model used was

\[ CH_4(t) = CH_4_{\text{max}} \times \exp \left(- \exp \left(\frac{k_{\text{max}} \times e}{CH_4_{\text{max}}}(\lambda - t + 1)\right)\right) \]

where \( CH_4(t) \) is the cumulative methane production (NmL g VS⁻¹) as a function of time (t in days), \( CH_4_{\text{max}} \) is the maximum methane production (i.e. the methane potential of the sample), \( k_{\text{max}} \) is the maximum methane production rate (NmL g VS⁻¹ day⁻¹), \( \lambda \) is the lag phase (days), and \( e \) is Euler’s number. The parameter \( k_{\text{max}} \) was normalized to the amount of VS added. The parameter estimation was performed by nonlinear least squares minimization (Nelder-Mead method) using the optimx package in R. The model parameters were evaluated by plotting the model together with the data, as well as plotting the residuals and the residual distribution. For the CTMP, TMP and raw fibres, the modified Gompertz model could not adequately capture the initial dynamics of methane formation, in which case the methane production rate between each measuring point was calculated by the slope of a linear function and the maximum value was reported as \( k_{\text{max}} \).

2.3. Solid-state and solution-state NMR

Metal ions in heterogeneous samples interfere with the NMR analyses of organic matter so fibre samples were treated with EDTA to remove metals that might be present. After freeze-drying, 800 mg of each sample was suspended in 40 mL EDTA solution with the concentration of 8 g L⁻¹ and placed on a platform shaker at 200 RPM for 1 h. Next, the samples were centrifuged at 10 000 × g for 10 min and the supernatant was removed. The pellets were re-suspended in 50 mL of milliQ water to remove any remaining EDTA and were centrifuged once more. Lastly, the pellets were freeze-dried and stored at room temperature until further analyses.

13C CP/MAS analyses were performed on a Bruker Avance III 500 MHz spectrometer equipped with a 4 mm MAS probe and a Bruker SamplePro solid-state sample changer. A 1 ms contact-pulse with a ramped ¹H amplitude (50–100%) and a ¹H decoupling strength of 80 kHz were used. 3500 scans were collected with a relaxation delay of 15s, resulting in an experimental time of approximately 1 h per sample. Spectra were normalized to a constant sum and placed in a data matrix for subsequent multivariate analysis using Matlab 2014b (Mathworks Inc.). Principal Component Analysis (PCA) was performed in SIMCA 14.1 (Umetrics).

Sample preparation for HSQC NMR analysis was performed as described by Shakeri Yekta et al. [19]. 2D ¹H–¹³C HSQC NMR spectra were recorded at 298 K on a Bruker Avance III HD 600 MHz spectrometer equipped with a BBO cryoprobe and a SampleJet sample changer. 16 scans were recorded for each of the 256 t₁ increments and spectra were calibrated using the residual DMSO peak (δH = 2.49 ppm and δC = 39.5 ppm). All spectra were phase-corrected manually and processed with Topspin 3.2 (Bruker Biospin, Germany). As CP-MAS and HSQC NMR spectroscopy are inherently qualitative, the NMR results were used to qualitatively analyse and compare the relative distribution of different molecular structures of the organic matter among the samples.

3. Results and discussion

3.1. Effects of the pulping process

The BMP tests showed that fibre residues from different pulping processes carried very different methane potentials (Fig. 2). Sulphite-, kraft-, NSSC-, and CTMP-pulped fibres showed methane potentials of 400 ± 17, 390 ± 6, 280 ± 2, and 160 ± 8 NmL CH₄ g VS⁻¹, respectively. The lowest methane potential of 50 ± 1 NmL CH₄ g VS⁻¹ was obtained from raw fibres. Fibres from TMP gave a methane potential lower than the inoculum control, which indicates an inhibition of the AD process. The methane production from kraft and sulphite fibres was relatively fast, with \( k_{\text{max}} \) ranging 130–200 NmL g VS⁻¹ day⁻¹ (Table 3). There was a short lag phase (\( \lambda \)) before methane production started, ranging 0.8–1.2 days for kraft and sulphite fibres. The methane production rate for NSSC was lower, \( k_{\text{max}} = 70 \) NmL g VS⁻¹ day⁻¹, with a lag phase of 1.4 days (Table 3), which together with the significantly lower (\( p < 0.05 \)) methane potential of 280 ± 2 NmL CH₄ g VS⁻¹, demonstrated that NSSC-pulped fibres were more difficult to degrade than chemically pulped fibres. The modified Gompertz model could not capture all the features of the degradation dynamics for CTMP- and TMP-pulped fibres nor for raw softwood fibres. In particular, the degradation appears to follow a two-phase pattern for CTMP-processed unbleached hardwood, TMP-

| Pulping process | Unbl. | P | ECF | TCF |
|-----------------|-------|---|-----|-----|
| Kraft           | Hw/Sw | Hw/Sw | Hw/Sw | Hw/Sw |
| Sulphite        | Hw/Sw | Hw/Sw | Hw/Sw | Hw/Sw |
| CTMP            | Hw/Sw | Hw/Sw | Hw/Sw | Hw/Sw |
| NSSC            | Hw    | Hw    |       | Hw/Sw |
| TMP             | Sw    |       | Sw    | Sw    |
| Raw fibre       | Hw/Sw | Hw/Sw | Hw/Sw | Hw/Sw |
The presence of easily degradable sugars such as glucose or cellobiose caused by the production of cellulases for the cellulose degraders, in this investigation, an increase in cellulase activity and a concomitant increase in biogas production could not be observed until after three days [21]. That the CTMP and TMP fibres were more difficult to degrade with a secondary lag phase, followed by colonization and degradation of larger, more recalcitrant fibres. In part, this could be coupled to a feedback inhibition in the production of cellulases for the cellulose degraders, caused by the presence of easily degradable sugars such as glucose or cellobiose (reviewed by [20]). After the depletion of such sugars, cellulose degraders in anaerobic systems generally colonize cellulose fibres by adhering to the surface of the fibres via complexed cellulase systems called cellulosomes [20]. The reasoning that such colonization of cellulose fibres could be the explanation to the two-phase pattern seen in this paper is supported by another study, where anaerobic microorganisms from an AD reactor were suspended in a buffer/mineral medium deficient in easily degradable sugars together with filter paper. In this investigation, an increase in cellulase activity and a concomitant increase in biogas production could not be observed until after three days [21]. That the CTMP and TMP fibres were more difficult to degrade was supported by the estimated $k_{max}$ being much lower for CTMP and TMP fibres (5–30 and 3–20 Nml g VS$^{-1}$ day$^{-1}$, respectively) compared to the range of chemically pulped fibres (130–200 Nml g VS$^{-1}$ day$^{-1}$; Table 3).

TMP and CTMP are mechanical pulping techniques that result in a high fraction of lignin remaining in the fibres after pulping [22]. Lignin in its native form has a very low degradability during anoxic conditions [23], since anaerobic microorganisms generally lack the oxidative enzymes and pathways required for depolymerization of high molecular-weight lignin [24]. This means that the microorganisms cannot use the lignin as a substrate to any accountable extent in biogas processes and have low access to the lignin-enclosed hemicellulose and cellulose. Thus, the lignin content most likely explains the low potentials observed for the TMP and CTMP fibres. This was supported by the chemical analyses showing relatively high lignin contents of 32% and 25–28% of dry weight for unbleached TMP and CTMP fibres, respectively, which is comparable with the lignin content of the raw fibres (Appendix B). Still, some anaerobic bacteria have been identified that are able to depolymerize lignin, and if the lignin is modified or treated, it becomes more easily degradable by anaerobic microorganism (reviewed by Khan & Ahring [24]), and could thereby be a contributing factor to the higher methane potential observed for the CTMP fibres compared to TMP. The kraft and sulphite process, and to some extent the NSSC process, utilizes chemicals at high temperatures and pressures to break up and dissolve the lignin. This removes most of the lignin in kraft fibres (3% remaining, Appendix B), and is likely the main explanation for the higher methane potentials obtained for these fibres. The removal of lignin was also clearly shown in the NMR analysis (see section 3.1.1). Furthermore, chemically modified lignin has been shown to have higher degradability than naturally occurring lignin during AD [25], and might also contribute to a higher methane potential for chemically treated fibres. Another important factor is the accessibility of the cellulose. One purpose of lignin in plants is to provide a protective barrier against microbial degradation, and its removal would not only increase the more easily biodegradable portion of the fibres, but also give enzymes better access to the cellulose (reviewed by Monlau et al. [26]). However, even though CTMP pulping did not remove any considerable amount of lignin compared to the raw fibres (28% and 29% remaining, respectively; Appendix B), the pulping likely increased the accessibility of the hemicelluloses and celluloses of the fibres by breaking up the lignin structures. This explains the higher methane potentials for these fibres compared to raw fibres.

### 3.1.1. NMR spectroscopy reveals differences in chemical composition

CP/MAS spectra of kraft, CTMP and TMP fibres from unbleached softwood are shown in Fig. 3. Peaks from all major wood components are identified and annotated accordingly. Lignin is identified by presence of broad peaks in the aromatic spectral region (110–160 ppm) and a more well-defined peak from the methoxy group at 65 ppm [27]. Peaks from cellulose are observed at 105 ppm (C1), 89 ppm (C4 from crystalline cellulose), 84 ppm (C4 from amorphous cellulose), 70–75 ppm (C2, C3 and C5) and 66–62 ppm (C6) and are often the most prominent peaks in spectra of lignocellulosic materials [28]. Peaks from hemicellulose are mostly obscured by the dominant cellulose peaks but one distinct spectral feature originating from hemicelluloses is the bump on the cellulose C1 peak observed at 101 ppm [29]. The peak at a chemical shift of 21 ppm originates from acetyl groups found in hemicelluloses [28]. Comparison of the NMR spectra demonstrates that part of the lignin fraction is still present in CTMP and TMP fibres while essentially no lignin is left in kraft fibres. These observations corroborate the results from Klason lignin analysis (Appendix B). In addition, hemicellulose is efficiently removed by the kraft process, as evident from the disappearance of both the resonance adjacent to cellulose C1 peak at 101 ppm.
Table 3
The estimated parameter values obtained after non-linear regression to biochemical methane potential (BMP) experimental data of the fibre samples. $CH_{A,max}$ is the maximum methane production rate (Nml g VS$^{-1}$ day$^{-1}$) predicted by the model, $k_{max}$ the maximum methane production rate (Nml g VS$^{-1}$ day$^{-1}$), $\lambda$ is the lag phase (day), and $k_{max}$ (\Delta y/\Delta t) is the maximum methane production rate estimated based on the gradient between two measuring points. For abbreviations, see Table 2. Kraft pulp was sampled from three different mills, denoted a–c, and samples marked with an asterisk were fibres collected from the wastewater. Visual evaluation of the model fits (plots of model, residuals, residual distribution) is commented on under Notes.

| Sample                | $CH_{A,max}$ (ml g VS$^{-1}$) | $k_{max}$ (Nml g VS$^{-1}$ day$^{-1}$) | $\lambda$ (day) | $R^2$ | $k_{max}$ (\Delta y/\Delta t) Notes |
|-----------------------|-------------------------------|--------------------------------------|-----------------|-------|-------------------------------------|
| Kraft-Sw-Unbl*        | 380                           | 130                                  | 1.2             | 0.98  |                                    |
| Kraft-Hw-Unbl*        | 380                           | 130                                  | 1.2             | 0.98  |                                    |
| Kraft-Sw-Unbl          | 380                           | 170                                  | 0.9             | 1.00  |                                    |
| Kraft-Sw-TCF          | 380                           | 170                                  | 0.8             | 1.00  |                                    |
| Kraft-Sw-Unbl*        | 380                           | 200                                  | 1.1             | 1.00  |                                    |
| Kraft-Sw-ECF          | 380                           | 200                                  | 0.8             | 1.00  |                                    |
| Sulphite-Sw-Unbl      | 380                           | 170                                  | 1.0             | 0.99  |                                    |
| Sulphite-Sw-TCF       | 380                           | 180                                  | 0.9             | 0.99  |                                    |
| Sulphite-Hw-Unbl      | 380                           | 150                                  | 1.0             | 0.99  |                                    |
| Sulphite-Hw-TCF       | 380                           | 190                                  | 0.8             | 0.99  |                                    |
| NSSC-Hw               | 280                           | 70                                   | 1.4             | 0.98  |                                    |
| CTMP-Hw-Unbl*         | 280                           | 50                                   | 2.0             | 0.91  | 30 (1)                              |
| CTMP-Hw-P*            | 280                           | 30                                   | 3.0             | 0.95  | 20                                   |
| CTMP-Sw-Unbl          | 130                           | 6                                   | 7.8             | 0.97  | 5 (2)                               |
| CTMP-Sw-P             | 160                           | 8                                   | 5.7             | 0.95  | 7 (2)                               |
| CTMP-Sw-P*            | 160                           | 10                                  | 6.6             | 0.94  | 8 (2)                               |
| TMP-Sw-Unbl*          | 260                           | 16                                  | 51.8            | 0.01  | 3 (3)                               |
| TMP-Sw-P*             | 120                           | 20                                  | 2.6             | 1.00  | 20 (1)                              |
| Raw-fibre-Sw          | 40                            | 1                                   | 6.0             | 0.83  | 3 (2)                               |
| Raw-fibre-Hw          | 210                           | 15                                  | 3.2             | 0.90  | 8 (2)                               |

(1) Poor fit to data.
(2) Poor fit to data, BMP not completed.
(3) Unable to fit to data, inhibition of AD process.

and the acetyl peak at 21 ppm. The $^{13}$C CPMAS spectra of bleached and unbleached TMP revealed several peaks in the aliphatic region (20–40 ppm) that were not present in any of the other spectra, with the exemption of the acetyl peak.

To get an overview of the chemical compositions of all fibre samples used in this study and relate that to the BMP results, a PCA was performed on the solid-state $^{13}$C CPMAS NMR spectra of all samples. A clear separation of mechanically pulsed fibres and chemically pulsed fibres was observed along the first principal component (explaining 88% of the variation; see Fig. 4a), implying that the pulping technique was the most important factor explaining the difference in chemical composition among the fibre samples. The loading plot (Fig. 4b) shows that the variation was largely related to the differences in aromatic and methoxyl structures of lignin in the fibre samples, with the chemically pulped fibres having lower intensities of lignin-derived $^{13}$C resonances (negative peaks in the aromatic spectral region 110–160 ppm and negative methoxy peak at 56 ppm). The hemicellulose peak at 101 ppm and the acetyl peak at 21.5 ppm are also negative, indicating more effective removal of hemicellulose in the chemically pulped fibres.

The second component in Fig. 4 (i.e. t2) separates the softwood (Sw) samples from the hardwood (Hw) samples, highlighting the differences in organic matter properties of the fibres in relation to the raw material used. However, this only accounts for 6% of the variation in NMR spectral data among the samples (Fig. 4). A detailed analysis of the loadings of the second principal component was not attempted as it showed a complex pattern (not shown). However, a positive peak was observed at 146 ppm, originating from guaiacyl (G), reflecting the difference in lignin composition between hardwood and softwood discussed below.

3.2. Effect of raw material

Comparisons of the methane potential of fibre from hardwood and softwood used in the same pulping process were specifically carried out for the Kraft, sulphite, and CTMP processes, as well as for raw wood (aspen and spruce). This comparison was not performed on the TMP and NSSC processes as these pulp mills did not shift their production between softwood and hardwood pulp. There were no significant differences in methane potentials for hardwood and softwood fibres from the Kraft or sulphite processes, which yield pulp with very low lignin content (Fig. 5). Kinetic modelling of the BMP data indicated a faster degradation of hardwood compared to softwood for Kraft fibres, with a $k_{max}$ of 180 and 130 Nml g VS$^{-1}$ day$^{-1}$, respectively, but a slower degradation of hardwood compared to softwood for sulphite fibres (150 compared to 170 Nml g VS$^{-1}$ day$^{-1}$, Table 3). Previous studies have indicated that available surface area is an important factor for enzymatic degradation [31], and as hardwood fibres are shorter and thinner than softwood fibres [22], a higher methane potential and/or a faster degradation might be expected for hardwood fibres. However, there seem to be conflicting results in the literature regarding pulp fibre length. Mooney et al. [32] demonstrated a faster and higher digestibility of refined, shorter fibres of a softwood Kraft pulp compared to untreated, longer fibres, whereas Del Rio et al. [33] could not show any difference in enzymatic degradation of pre-treated and size-fractionated softwood fibres. Within the scope of this study, no difference in methane production rates or potentials could be observed for chemically pulped fibres originating from hardwood or softwood. Thus, shifts in raw material during Kraft or sulphite pulp and paper production would presumably have limited effect on a full-scale biogas process.

AD of raw fibres and CTMP pulped hardwood fibres gave rise to significantly ($p < 0.01$) more methane than the corresponding softwood fibres, i.e. 240 ± 8 compared to 50 ± 1 Nml CH$_4$ g VS$^{-1}$ for raw fibres and 300 ± 10 compared to 160 ± 8 Nml CH$_4$ g VS$^{-1}$ for CTMP fibres (Fig. 5). The kinetic models indicated faster methane production rates, with an estimated $k_{max}$ of 20 compared to 7 Nml g VS$^{-1}$ day$^{-1}$ for CTMP (bleached) and 8 compared to 3 Nml g VS$^{-1}$ day$^{-1}$ for untreated fibres (Table 3, Appendix B). One contributing factor to these differences is likely that softwood (spruce) used at the mill naturally has a higher lignin content than hardwood (27%, Appendix C, compared to 20–22% [26]). Furthermore, overlays of $^{13}$C CPMAS spectra of soft- and hardwood fibres – either raw (Fig. 6a) or CTMP-pulped (Fig. 6b) – show clear dissimilarities in intensities of lignin-derived $^{13}$C peaks ($\delta C$: 105–155 ppm). This is a reflection of the difference in their lignin composition, where hardwood lignin contains syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H) monomers while softwood lignin contains almost exclusively G monomers [34]. The main bond formed between the lignin monomers is the relatively weak aryl-ether bond (i.e. $\beta$-O-4 bond), which makes up 35–60% and 50–70% of the bonds in softwood and hardwood, respectively [35,36]. The lower occurrence of this bond in softwood is coupled to the ability of $\gamma$-type lignin to form the more chemically stable C-C bonds between the G units, whereas C-C bonds cannot be formed between S units [26,35]. For this reason, hardwood fibre lignin is more likely to be affected by the CTMP process, and the accessibility of the hemicellulose and cellulose for degradation is improved. In terms of AD, however, previous lab-scale BMP tests on ground wood samples revealed no clear degradation of lignin despite degradation times of more than 440 days [37,38]. This suggests that degradation of lignin in AD is not the explanation for the observed difference in methane production between hardwood and softwood.

In another study, AD of ground hardwood (sycamore) showed a faster methane production than from softwood (pine) in BMP tests carried out for 27 days, and at the same time a higher removal of xylan [39]. Generally, hardwood has a higher content of xylose and softwood has a higher content of mannose [40,41]. In a study by De la Cruz et al. [38], the xylose content in hardwood during AD was decreased by 30% in BMP tests, whereas the sugar content of the softwood sample (mainly
mannose) was unaffected. This indicates that xylose is more easily degraded and may explain why hardwood fibres produced methane more quickly than did softwood fibres in this study.

Furthermore, hardwood hemicellulose is acetylated to a larger extent compared to softwood hemicellulose (reviewed by Pawar et al. [42]). The presence of acetyl groups in xylans and mannans may hinder enzymatic hydrolysis of polysaccharides [43], and selective deacetylation has been shown to increase enzymatic (cellulase, cellobiase) digestibility of wood [44,45]. The decrease in intensity of the acetyl peak observed in this study (Fig. 6) for hardwood – but not for softwood – after pulping may therefore have facilitated hydrolysis and partly explains the higher methane potential of hardwood CTMP fibres compared to softwood CTMP fibres during AD.

### 3.3. Effect of bleaching

Like the results for raw material, bleaching had no significant effect on the methane potentials of the fibres from kraft and sulphite pulping (Fig. 7). Further, there was no difference in $k_{\text{max}}$ for kraft fibres, whereas for sulphate fibres, bleaching had a positive effect, particularly for hardwood, with a $k_{\text{max}}$ of 190 compared to 150 Nml g VS$^{-1}$day$^{-1}$.

There was a significant difference ($p < 0.01$) in methane potential for unbleached and bleached softwood CTMP fibres (160 ± 8 and 210 ± 12 Nml CH$_4$ g VS$^{-1}$, respectively), and the estimated $k_{\text{max}}$ indicated a slightly faster degradation of bleached softwood CTMP fibres (8 Nml g VS$^{-1}$day$^{-1}$) compared to 5 Nml g VS$^{-1}$day$^{-1}$, Table 3). The BMP tests for these two samples were, however, not continued until methane production ceased completely, meaning that the total methane produced could eventually be more similar. In the context of using the fibres for biogas production, it is not feasible to allow the BMP test to continue longer than 90 days.

For hardwood CTMP fibres, bleaching had no significant effect on methane potential, with 290 ± 8 and 300 ± 10 Nml CH$_4$ g VS$^{-1}$ for unbleached and bleached, respectively, whereas $k_{\text{max}}$ was higher for the unbleached fibres (30 Nml g VS$^{-1}$day$^{-1}$) compared to 20 Nml g VS$^{-1}$day$^{-1}$, Table 3). One possible explanation for the slower degradation could be the presence of residual bleaching chemicals or wood compounds that have been released during bleaching. This is supported by findings from another mill using the same bleaching chemical (peroxide). Here CTMP wastewater inhibited methane formation during BMP tests of wastewater generated during the production of bleached pulp, whereas the corresponding wastewater for unbleached pulp showed no such effect (Appendix D).

The hemicellulose composition of the CTMP softwood fibres was altered by bleaching as evident by the different appearance of the hemicellulose C1 region in the $^{13}$C CPMA spectra ($\delta_{C}$: 95–102 ppm); see Fig. 8a. The dominant hemicellulose in softwood is galactoglucomannan and a significant portion of this hemicellulose is present even after bleaching, as evidenced by the presence of the C1 signal from mannose at $\delta_{C}$: 102 ppm (Fig. 8a). However, some of the hemicellulose has been removed, observed in the reduction in $^{13}$C CPMA signal intensity around 98 ppm as well as in several other carbohydrate peaks between 60 and 91 ppm. Exactly how the hemicellulose is modified is difficult to deduce but a plausible explanation may be the removal of the galactose units attached to the main chain of galactoglucomannan. Deacetylation during bleaching is also apparent as the acetyl signal (21 ppm) completely disappeared (Fig. 6). Interestingly, bleaching did not change the lignin content of CTMP softwood fibres (Appendix C), yet a higher methane yield was achieved for this sample compared to unbleached CTMP fibres. This suggests that the removal of acetyl groups and the altered hemicellulose structure made the softwood fibres more susceptible to degradation, but the mechanistic details are not clear.

The lignin content of hardwood CTMP fibres decreased from 25% to 18% (Appendix C), which is also visible in Fig. 8b as a decrease in the peak representing the methoxy group in lignin ($\delta_{C}$: 56 ppm). Furthermore, the acetyl groups that remained after pulping were removed by bleaching, whereas the decrease in hemicelluloses observed for softwood was not apparent for hardwood (Fig. 8b). Despite the decrease in lignin and acetyl groups, neither the rate of degradation nor the methane potential of the bleached hardwood sample was improved; instead $k_{\text{max}}$ was lower than for the unbleached fibres (20 compared to 30 Nml g VS$^{-1}$day$^{-1}$, Table 3). Possibly, the CTMP pulping of hardwood fibres and the removal of acetyl groups improved the accessibility/degradability of the fibres to such an extent that additional modifications to the hemicellulose by bleaching did not further improve the degradability.

In a study by Larsson et al. investigating the methane potential of bleached CTMP wastewaters, the concentrations of total organic carbon (TOC) and acetic acid were about twice as high in wastewaters from
hardwood pulping as raw material (3800–4600 mg TOC L\(^{-1}\), 60–70 mmol acetic acid L\(^{-1}\)) compared to softwood (2100–2800 mg TOC L\(^{-1}\), 20–30 mmol acetic acid L\(^{-1}\)) [47]. This corresponds well to the disappearance of the acetyl peak in Fig. 8a and b, and the proportionally higher intensity of the acetyl peak in hardwood before bleaching. Still, the higher TOC in the hardwood CTMP wastewater indicates that other organic structures are removed from the wood as well.

For TMP, the fact that unbleached fibres produced less methane than the inoculum control (as evident by the negative methane potential) suggests that AD was inhibited. Similarly, inhibition of the AD process was observed during the initial days of BMP tests of both pulping and bleaching wastewater from the same mill, confirming that inhibitory compounds are released during these processes. Bleached TMP fibres, however, did not demonstrate an inhibition of degradation, resulting in a methane potential of 130 ml CH\(_4\) g VS\(^{-1}\) (Fig. 7) and an estimated \(k_{\text{max}}\) of 20 Nml g VS\(^{-1}\) day\(^{-1}\) (Table 3). The 2D HSQC spectrum (Fig. 9) indicated the presence of unsaturated aliphatic CH bonds in the unbleached fibre sample in addition to a large number of peaks in the aliphatic region in both bleached and unbleached TMP fibres. After bleaching, a large number of aliphatic peaks still persist, whereas the peaks from the double bonds are not present. Furthermore, aromatic structures not associated with lignin appear in the HSQC spectrum of unbleached TMP fibres, but those peaks are not present after bleaching. A previous chemical analysis of the bleaching wastewater at the TMP mill showed the presence of extractives such as resin acids and triglycerides (50 mg l\(^{-1}\) and 72 mg l\(^{-1}\), respectively). As the TMP fibres were collected from the wastewaters, these compounds were likely present in the sample and could be the cause of the inhibition observed during the BMP test; the release of these compounds into the wastewater during bleaching explains why the bleached TMP fibre sample did not give rise to any apparent inhibition.

### 3.4. Pulp and paper mill primary sludge

An important consideration is that this study was based on pulp fibres sampled from the product line or the pulp washing. The primary sludge from the primary clarification at the mill would likely contain residue compounds from the different process steps, such as CaCO\(_3\) (caused by poor chemical recovery) or fillers from the paper production [48]. The inorganic chemicals would reduce the proportion of VS in the primary sludge and thereby also lower the methane potential of the primary sludge per ton of TS. Within this study, BMP tests of primary sludge from three different kraft mills were performed, demonstrating a considerable variability in both VS content and methane potential.
Fig. 5. Accumulation of methane minus control in BMP tests of hardwood (Hw) and softwood (Sw) from different pulping processes (before bleaching). CTMP = chemical thermo-mechanical pulping.

Fig. 6. $^{13}$C CPMAS NMR spectra on hardwood (Hw; black) and softwood (Sw; grey) in a) raw fibres and b) fibres produced with CTMP. $N = 3$ for both CTMP samples. S and G refers to syringyl (S) and guaiacyl (G) units in lignin, with numbers referring to the different carbon positions in each structure. See Fig. 3 for assignment of the non-lignin peaks. Lignin assignment adapted from Almendros et al. [46].
Fig. 7. Accumulation of methane minus control in BMP tests on unbleached/bleached fibres from different pulping processes. See Fig. 1 for abbreviations. All samples are softwood, unless denoted hardwood (Hw).

Fig. 8. $^{13}$C CPMAS spectra of bleached (black) and unbleached (grey) fibres produced by CTMP using a) softwood (Sw) or b) hardwood (Hw). The region where hemicellulosic C1 carbons appear is marked with an asterisk [29].
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Evidently, the higher VS content of the first sample resulted in a higher methane potential in the sludge compared to the primary sludge from the other two mills. Furthermore, the second mill also produced 20% NSSC pulp, which, in line with the results presented for pulp fibres above, reduced the methane potential of the primary sludge. Thus, site-specific conditions may affect the amount of methane that can be extracted from the available primary sludge, which should be considered prior to any full-scale implementation.

4. Conclusions

In summary, fibres from kraft and sulphite pulping would be suitable substrates for AD, demonstrating high methane potentials (400 ± 17 and 390 ± 6 Nml CH$_4$ g VS$^{-1}$, respectively). These fibres also had the highest rate of methane production (130–200 Nml g VS$^{-1}$ day$^{-1}$), most likely due to the efficient removal of lignin during the pulping processes. Furthermore, the methane potential for these fibres did not differ during shifts in raw materials used or shifts in production between unbleached and bleached pulp, the methane production at a full-scale AD plant would likely remain stable despite frequent variations in these operational conditions. Thus, our results strongly promotes the application of biogas production from kraft and sulphite primary sludge, and would result in a considerable methane production worldwide as chemical pulping represents 82% of the world’s virgin pulp production (54% if including recovered fibre pulp [49]). CTMP-pulped fibres would, however, give rise to a substantial variation in methane production during shifts in raw material and between unbleached/bleached qualities of pulp. Here the methane potentials were 300 ± 10 and 160 ± 8 Nml CH$_4$ g VS$^{-1}$ for CTMP hardwood and softwood pulp, respectively. This means that the application of CTMP fibres for methane production would vary from site to site, depending on the ratio of hardwood to softwood that is used at each mill and how often the mill shifts between these different raw materials during pulp production. The mechanism behind the higher degradability in hardwood was likely related to a lower lignin content with more β-O-4 bonds that are easier to break compared to softwood. Furthermore, the hemicellulose was more affected by pulping, possibly by a higher degree of deacetylation of the hardwood xylan, which made it more susceptible to degradation. In addition, bleaching had a positive effect on the methane potential for softwood CTMP fibres, where the acetyl groups of softwood CTMP fibres were removed upon bleaching and the composition of the hemicellulose was affected, likely improving fibre degradability. For TMP fibres, the presence of extractives originating from the wood inhibited biogas production, while bleaching alleviated the inhibitory effects of these compounds, and enabled the conversion of fibres to biogas during AD.

Acknowledgements

The authors wish to thank the personnel at SCA and BillerudKorsnäs and Xu-Bin Truong at Scandinavian Biogas Fuels AB for their assistance within this study. The authors would also like to thank NMR for Life, a project funded by the Knut and Alice Wallenberg foundation. The study was funded by the Swedish Energy Agency (Grant No. 32802-2), Scandinavian Biogas Fuels AB, Föyry AB, BillerudKorsnäs AB, SCA, Fiskeby Board AB and Purac AB. It was also supported by the Swedish Research Council Formas [Grant number: 2016-01054] and Biogas Research

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Table 4

| Methane potential, organic content (volatile solids (VS) as per cent of total solids (TS)) and the approximate composition of the primary sludge from three kraft pulp and paper mills. | CH$_4$ (Nml g VS$^{-1}$) | VS (% of TS) | Composition |
|---|---|---|---|
| | 310 ± 4 | 90 | 100% kraft |
| | 170 ± 6 | 85 | 80% kraft, 20% NSSC |
| | 200 ± 13 | 70 | 100% kraft |

(Table 4) Evidently, the higher VS content of the first sample resulted in a higher methane potential in the sludge compared to the primary sludge from the other two mills. Furthermore, the second mill also produced 20% NSSC pulp, which, in line with the results presented for pulp fibres above, reduced the methane potential of the primary sludge. Thus, site-specific conditions may affect the amount of methane that can be extracted from the available primary sludge, which should be considered prior to any full-scale implementation.

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Fig. 9. 2D $^1$H–$^{13}$C HSQC spectra from unbleached (black) and bleached (cyan) thermo-mechanically pulped (TMP) softwood (Sw) fibres. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2019.105325.
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