

Biogas Production from *N*-Methylmorpholine-*N*-oxide (NMMO) Pretreated Forest Residues

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Abstract Lignocellulosic biomass represents a great potential for biogas production. However, a suitable pretreatment is needed to improve their digestibility. This study investigates the effects of an organic solvent, *N*-Methylmorpholine-*N*-oxide (NMMO) at temperatures of 120 and 90 °C, NMMO concentrations of 75 and 85 % and treatment times of 3 and 15 h on the methane yield. The long-term effects of the treatment were determined by a semicontinuous experiment. The best results were obtained using 75 % NMMO at 120 °C for 15 h, resulting in 141 % increase in the methane production. These conditions led to a decrease by 9 % and an increase by 8 % in the lignin and in the carbohydrate content, respectively. During the continuous digestion experiments, a specific biogas production rate of 92 NmL/gVS/day was achieved while the corresponding rate from the untreated sample was 53 NmL/gVS/day. The operation conditions were set at 4.4 gVS/L/day organic loading rate (OLR) and hydraulic retention time (HRT) of 20 days in both cases. NMMO pretreatment has substantially improved the digestibility of forest residues. The present study shows the possibilities of this pretreatment method; however, an economic and technical assessment of its industrial use needs to be performed in the future.

Keywords Biogas · Anaerobic digestion · Forest residues · Batch experiment · Continuous experiment

Nomenclature

NMMO	<i>N</i> -Methylmorpholine- <i>N</i> -oxide (NMMO)
OLR	organic loading rate
HRT	hydraulic retention time
VFA	volatile fatty acids
VS	volatile solids
TS	total solids
SD	standard deviation

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HPLC	high performance liquid chromatography
GC	gas chromatography
CSTR	continuous stirred tank reactor
WWTP	wastewater treatment plant

Introduction

As a consequence of the enormous wood exploitation, a large amount of forest residues, mainly consisting of leaves, small branches, and bark, is generated. Up to date, the majority of these forest residues are abandoned in situ, thus originating important environmental problems. Among these problems, soil acidification (due to the accumulation of organic matter) and increased risk of forest fires, especially during dry periods with high temperatures can be mentioned [1].

Forest residues are one of the most abundant lignocellulosic waste streams in Sweden with 1.6 million tons total solids (TS) per year reported for 2008. This amount is predicted to more than double by the year 2018 [2]. This makes forest residues a potential biomass for biogas production and an energy production on a scale of 59 TWh/year in Sweden [3]. There is a large demand for alternative fuels produced from renewable resources worldwide especially for the transport sector, and biogas is one of the alternatives which can be used. However, in order to meet these increasing requirements, new sources of substrates are needed to be utilized for biogas production [4]. Lignocellulosic biomass, such as forest residues, is primarily composed of cellulose, hemicelluloses, and lignin. These kinds of materials can serve as an inexpensive substrate for biofuel production, avoiding the moral dilemma connected with the utilization of potential food resources [5]. However, the enzymatic conversion of the cellulose and hemicelluloses in lignocelluloses is slow if the biomass is not exposed to some kind of pretreatment.

Several pretreatment methods have been investigated, including ammonia fiber explosion (AFEX), wet oxidation, and liquid hot water (LHW), among others, which are shown to be more successful for agricultural and forestry residues. However, all these pretreatments performed on forest residues were carried out prior to ethanol production. Furthermore, none of these pretreatment methods are considered to be enough efficient today [6].

Hendriks and Zeeman [7] reviewed the effect(s) of several pretreatment methods on the three main parts of the lignocellulosic biomass to improve its digestibility. Steam, lime, LHW, and ammonia-based pretreatments are concluded to be pretreatments with high potential. Their main effects are dissolving hemicellulose and alteration in the lignin structure, providing an improved accessibility to the cellulose for hydrolytic enzymes. However, it was also concluded that many of these methods give rise to different inhibitory products, which especially in high concentrations can possibly be very harmful to microorganisms in anaerobic digestion. On the other hand, it was also concluded that during anaerobic digestion the microorganisms have a potential to adapt to these inhibitory products, when presented at very low concentrations.

Recent studies [8–10] used an organic solvent *N*-methylmorpholine-*N*-oxide (NMMO) for regeneration of cellulose in the industrial Lyocell process and show that this solvent has a great potential for the pretreatment of lignocelluloses. The melting point of this industrial solvent is about 70 °C, while it decomposes at temperatures higher than 130 °C. Consequently, most of the pretreatment studies with NMMO are performed between these temperatures [11].

Earlier studies focusing on NMMO treatment of lignocellulosic materials aimed either to improve the ethanol production rate [7–9] or to determine the effects of the treatment on biogas production through anaerobic batch digestion assays [10, 12].

Previous studies showed that the behavior of wood and cotton cellulose fibers pretreated with NMMO is highly affected by the water content in the solvent [13]. In a recent study, it was shown that pretreatment of cotton with NMMO corresponding to NMMO concentrations of 85, 79, and 73 % resulted in dissolution, ballooning, and swelling of the cellulose fibers [14]. All of the experiments were carried out at both 90 and 120 °C during treatment times of 0.5–15 h. The study showed that the dissolution (85 %) mode had the best effect on the following enzymatic hydrolysis of cellulose. However, the swelling (73 %) and ballooning (79 %) mode resulted in the highest yields of methane production during the following anaerobic digestion.

Another study on NMMO pretreatment of softwood spruce and hardwood oak with 85 % at 90, 110, and 130 °C for 1–3 h showed that the temperatures as well as the treatment times had significant effects on the performance of the following enzymatic hydrolysis [9]. Furthermore, pretreatment with 85 % NMMO at 130 °C for 1–15 h on lignocellulosic materials, such as spruce chips from the Swedish forests, triticale straw from the Swedish farmland, and rice straw from the Indonesian fields indicated that increasing the pretreatment time can improve the methane yield during the following anaerobic digestion [10].

All previous studies used batch digestion assays to determine the effects of different treatment conditions on methane yield and methane production rate. This study was performed to investigate the long-term effects of the NMMO treatment using continuous digestion experiments. The substrate utilized was forest residues in Sweden, which is a heterogeneous material with high lignin content. Biogas production from the treated vs. untreated materials were compared after different treatment conditions as well as at different operational conditions.

Experimental Section

Raw Materials

The forest residues were delivered by Norrskog (Östersund, Sweden). It was an inhomogeneous material consisting of a mixture of both spruce and pine with a high amount of bark. The material was first milled to 0.5–2 mm in size using a laboratory mill (Retsch SM100, Retch, Germany) prior to characterization, treatment, and digestion. The characterization of the untreated material showed that it consisted of 45.75 % lignin and 41.05 % carbohydrates.

NMMO Pretreatment

The NMMO pretreatments at different conditions were carried out using an industrial grade, 50 % w/w, NMMO solution obtained from BASF (Ludwigshafen, Germany). In order to concentrate the solution up to 75 and 85 %, a rotary evaporator (Laborata 20 eco, Heidolph, Germany), operating at a pressure of 0.10 bar and a maximum temperature of 130 °C was used. The pretreatments were performed in 5 L beakers containing 6 % forest residues in either 75 or 85 % NMMO solution. During the pretreatments, the reaction mixtures were heated in an oil bath at 120 or 90 °C for 3 and 15 h at atmospheric pressure, while mixing constantly with a mixer. After the pretreatments, the reaction was stopped by adding 1 L of boiling water to the beakers. The pretreated materials were then filtered and washed with hot tap water, which made it easier to dissolve and wash away the NMMO until no traces of NMMO was observed in the filtrate. The pretreated materials were stored at 6 °C until further use.

Biogas Production

Batch Digestion Experiments

The anaerobic batch digestion experiments were carried out at mesophilic (37 °C) conditions according to a method that was published earlier by Hansen et al. [15]. The inoculum used in mesophilic experiments was obtained from a large scale digester treating municipal wastewater sludge (Tekniska Verken, Linköping, Sweden). The batch assays were performed using sealed serum glass bottles with a volume of 118 ml, and all experimental setups were prepared in triplicates. All assay bottles contained 40 mL inoculum, pretreated or untreated forest residues as substrate in amounts to achieve a volatile solids (VS) ratio inoculum to substrate of 2:1 and tap water to give a final volume of 45 mL. To determine the methane production from the inoculum itself, blanks containing only inoculum and tap water without any substrate addition were also examined. In order to create anaerobic conditions and to avoid pH change, the headspace of each reactor was finally flushed with a gas mixture containing 80 % N₂ and 20 % CO₂. During the experimental period of 52 days, the reactors were shaken and moved around in the incubator once a day.

The production of methane was measured by taking gas samples regularly from the headspace, using a pressure-tight gas syringe. During the first 2 weeks, samplings and measurements were carried out in every third day, followed by once a week for the rest of the experimental period. The pH in the reactors was measured at the end of the experiment.

Semi-Continuous Anaerobic Digestion Experiments

The semi-continuous experiments were carried out at mesophilic conditions (37 °C) in 2 L glass digesters, equipped with plastic tubes protruding the top of the reactor and ending in the liquid phase; one for addition and withdrawal and one for the impeller. A rubber stopper with an outlet for gas covered the top of the reactor. Two experiments were performed in parallel; one with milled forest residues and one with NMMO-treated milled forest residues. Both digesters were fed with reject water and digested sludge obtained from a municipal wastewater treatment plant (WWTP) (Linköping, Sweden). These experiments continued for 118 days and were performed in two ways: fed batch during start up and thereafter, as semicontinuously fed digesters. Initially, 540 mL of digester sludge from a municipal WWTP together with 260 mL of reject water was added to a 2 L glass reactor. Forest residues (pretreated or untreated) together with reject water and digester sludge were then added daily to the digesters until they reached a working volume of 1,500 mL on day 26. Thereafter, the digesters were kept at a constant hydraulic retention time (HRT) of 20 days by daily withdrawal of digester fluid and addition of substrate, reject water, and digester sludge (90 % of organic loading rate (OLR) from forest residues) as explained above. The digesters were both initially loaded with 2.0 g VS/L/day. Every fifth day, the OLR was increased with 0.5 g VS/L/d until reaching 4.2 g VS/L/day. Due to process disturbances, the OLR was decreased to between 2.4 and 3.7 g VS/L during days 48–55. However, from day 56 to 118, the OLR was kept at 4.4 g VS/L. Because of the process disturbance, 350 mL digester fluid was removed from the digester and replaced with 100 mL digested sludge and 100 mL of reject water from the same source as described above, on day 49. In addition, from day 49 onward, the daily amount of reject water was decreased by 10 mL (to 21 mL) and the amount of digested sludge was increased by 10 mL (to 26 mL). Stirring was initially (up to day 12) performed with a magnet and thereafter, with a metal impeller at regular intervals, five times a day. Volatile fatty acids (VFAs), pH, TS, and VS in the digestate residue were measured weekly. The gas production was measured continuously using gas meters (own

design), working according to the gas displacement method. All gas volumes are given at standard conditions.

Analytical Methods

Total carbohydrate and lignin contents of the pretreated and untreated forest residues were determined according to the NREL procedures [16]. In these methods, a two-step acid hydrolysis with concentrated and diluted sulfuric acid was performed to release the sugars from the hemicellulose and cellulose fractions. The amount of different liberated sugars was measured afterward by HPLC (Waters 2695, Millipore, Milford, U.S.A.) equipped with a refractive index (RI) detector (Waters 2414, Millipore, Milford, U.S.A.) and an ion-exchange column (Aminex HPX-87P, Bio-Rad, U.S.A.) at 85 °C, using ultra-pure water as eluent with a flow rate of 0.6 ml/min. The acid-soluble and acid-insoluble lignin contents were analyzed using UV spectroscopy at 205 nm and after drying the material at 575 °C, respectively. All lignin and carbohydrate analyses were carried out in triplicates.

The methane and carbon dioxide in the anaerobic batch digestion series were analyzed as described by Teghammar et al. [17], using a gas chromatograph (Auto System, Perkin-Elmer, USA) equipped with a packed column (Perkin-Elmer, 6' × 1.8" OD, 80/100 Mesh, USA) and a thermal conductivity detector (Perkin-Elmer, USA) with the inject temperature of 150 °C. The carrier gas was nitrogen, operated with a flow rate of 20 ml/min at 60 °C. A 250- μ l pressure-tight gas syringe (VICI, Precision Sampling, Inc., USA) was used for gas sampling. The overpressure in the bottles caused by the excess gas was released through a needle following the gas analyses in order to avoid overpressure higher than 2 bar in the head space of the flasks. The methane content in the gas produced during the continuous stirred tank reactor (CSTR) experiments was not measured due to the low amount of gas being produced, which caused too large errors in the measurements. All the results are presented as gas volume at normal conditions (0 °C and atmospheric pressure) per kilogram VS.

Total solids and VS were determined by drying the samples to a constant weight at 105 °C and then igniting the dried material at 575 °C [18].

The VFAs obtained during the CSTR experiments was measured using GC-FID as described by Jonsson and Borén [19]. The pH was measured with a pH electrode (WTW Inolab, Germany).

Results

Effects of NMMO Pretreatment on the Composition of Forest Residues

The composition of forest residues before and after NMMO pretreatments at different conditions is presented in Table 1. The pretreatment with 75 % NMMO at 120 °C for 15 h increased the total carbohydrate content by 8 % (from 41 to 49 %) in comparison to the untreated forest residues. On the other hand, the pretreatment with 85 % NMMO for 3 h and at 120 °C increased the carbohydrate content from 41 wt% for untreated material to approximately 45 wt% for the treated materials. The decrease in the pretreatment temperature from 120 to 90 °C did not have a considerable effect, so the carbohydrate content remained at the same level as it was obtained for the untreated material. However, when the temperature was decreased from 120 to 90 °C, while all the other parameters for the pretreatment remained the same, i.e., 85 % NMMO and 3 h, a decrease by 3.5 % in the carbohydrate content was observed.

Table 1 Pretreatment condition, Methane production, Carbohydrates, lignin of untreated and NMMO- Pretreated forest residue

Pretreatment conditions		Methane production						
Temperature (°C)	NMMO (%)	Treatment time (h)	Total solids (%)	Volatile solids (%)	Total lignin (wt%)	Initial reaction rate		
						Total carbohydrates (%) (Nml/g VS/day) ^a	Yield (Nml/gVS _{added}) ^b	
120	75	15	30.5	29.69	36.48	49.05	4.27±1.5	100.5±16
120	85	3	26.77	26.04	38.67	45.3	3.65±1.0	109.5±20
90	85	3	27.33	26.48	40.55	41.8	2.75±0.6	87.68±17
Untreated	–	–	50.7	45.64	45.75	41.05	0.83±0.4	41.53±3.0

^a Reaction rate during the first 10 days with two standard deviations^b Accumulated methane gas produced per gram volatile solids after 52 days of incubation with two standard deviations

The NMMO pretreatment resulted in a decrease in total lignin content. The highest decrease in lignin was achieved when the forest residues were pretreated with 75 % NMMO at 120 °C for 15 h, reducing the lignin content by over 9 % (from 45.75 to 36.48 %). The pretreatment with 85 % NMMO for 3 h decreased the lignin content by 7 and 5 %, when the forest residue was treated at 120 and 90 °C, respectively.

Batch Digestion of NMMO Pretreated vs. Untreated Forest Residues

Anaerobic digestion of untreated forest residues resulted in 42 NmL CH₄/gVS_{added} (Fig. 1). Furthermore, the initial reaction rate of untreated forest residues obtained within the first 10 days of digestion was 0.83 NmL/gVS/d (Fig. 1 and Table 1). The pretreatment at 120 °C with 85 % for 3 h and with 75 % NMMO for 15 h had a positive effect on the methane production. The methane yield increased more than twofold, achieving up to 109 and 100 NmL CH₄/gVS_{added}, respectively. The initial reaction rate was also improved, achieving 4.27 and 3.65 NmL/g VS/day after 15 and 3 h pretreatment, respectively. The material pretreated with 85 % NMMO for 3 h and at 90 °C showed a slightly lower methane yield of 87 NmLCH₄/g VS_{added} and methane production rate of 2.75 NmL/g VS/day. The highest methane production rate, i.e., 4.27 NmL CH₄/gVS/day, was observed after pretreatment with 75 % NMMO at 120 °C for 15 h. Therefore, the forest residues pretreated at these conditions were further investigated during the CSTR experiments.

Anaerobic Digestion Forest Residues in Fed-Batch and Semi-Continuous Mode

The specific biogas production was 53 NmL/gVS/day ($n=63$; standard deviation (SD) ± 7) for digester 1 (Fig. 2), where untreated forest residues were included in the feed. In digester 2, where the treated forest residues were digested, specific biogas production of 92 NmL/gVS/day ($n=51$; SD ± 24) was obtained (Fig. 3). Both digesters were operating at maximum OLR of 4.4 gVS/L/day with HRT of 20 days. Under these conditions, the mean VS-reduction was 8 % (SD ± 4) for digester 1 and 20 % (SD ± 6) for digester 2. The pH was measured at between 7.4–7.8 in reactor 1 and between 7.1–7.6 in reactor 2 during the experiments (Fig. 3).

Acetate and propionate were the main VFAs detected, and the values varied both between the processes and over time. Starting on day 41, the amount of acetate and propionate

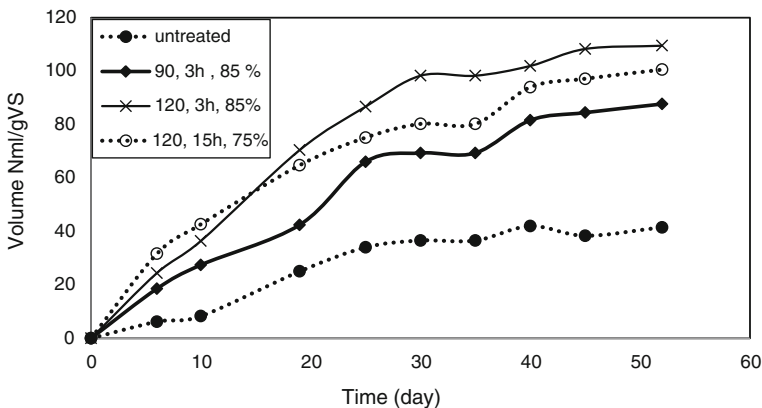


Fig. 1 Accumulated methane production on NMMO pretreated and untreated forest residue at mesophilic batch condition. The pretreatment conditions are described in the figure

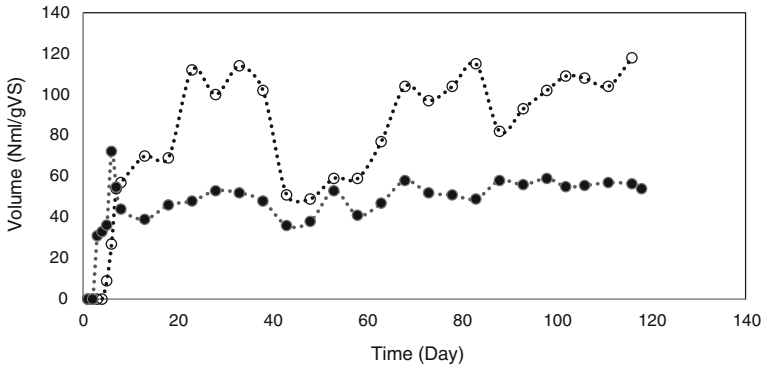


Fig. 2 Specific gas production for untreated forest residues (●...) and NMMO-treated forest residue (o...)

increased in reactor 2, reaching at most 7.1 mM acetate and 1.3 mM propionate on day 49. On day 51, both acetate and propionate started to decrease to concentrations lower than 0.6 mM. In reactor 1, both acetate and propionate was below 0.6 mM during the experimental period.

Discussion

In accordance with previous studies on NMMO-pretreated lignocellulosic materials, it was found that the pretreatment had positive effects, resulting in increased methane yields during the subsequent anaerobic batch digestion assays. A previous study on NMMO pretreatment of lignocellulosic materials such as spruce, rice straw, and triticale straw showed an increase between 400 and 1,200 % in the methane production after the treatment [10]. However, the

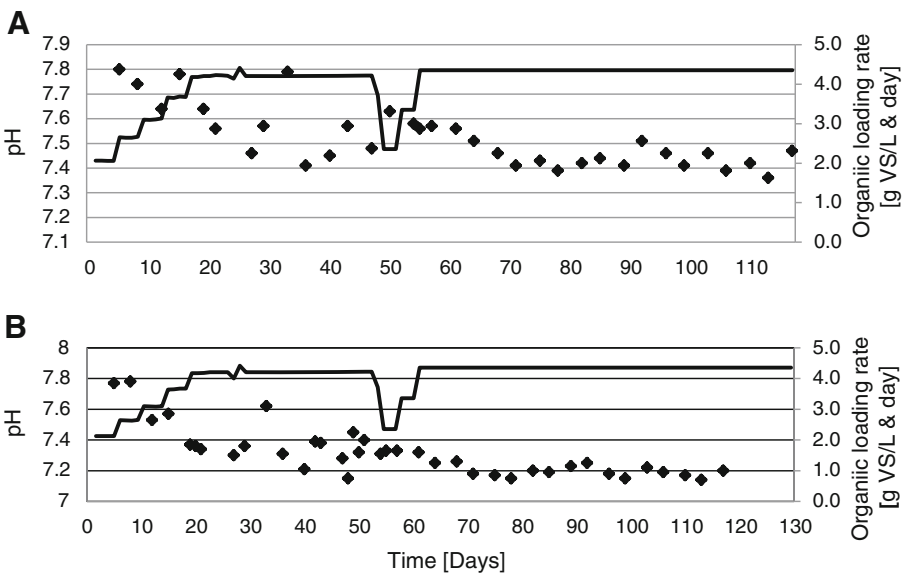


Fig. 3 pH (◆) and organic loading rate (—) for the reactors fed with **a** untreated forest residue and **b** NMMO-treated forest residues

substrates that were used there were homogenous with lower lignin content (between 19 and 29 wt%) compared to the heterogeneous forest residues with much higher lignin content of 46 wt%. The pretreatment (75 % NMMO, 120 °C for 15 h) decreased the total lignin content by more than 9 % and consequently, increased the total carbohydrates by 8 % resulting in an increase in methane production by 141 %.

The long-term effects of the most effective pretreatment conditions were further investigated in semicontinuous anaerobic digestion using pretreated vs. untreated forest residues as substrates. The results obtained in our study clearly demonstrate that a continuous biogas process can also be based on NMMO-treated forest residues with a low addition of supplemental material to keep the nutritional balance in the system. To our knowledge, this is the first stable continuous digestion of NMMO-treated forest residues to be shown.

The biogas yield was also improved from 53 to 92 NmL/gVS/day, during continuous digestion of untreated and treated forest residues, respectively (Fig. 2). The rapid increase in VFAs obtained in digester 2 is probably due to the higher level of organic material available for digestion in the NMMO-treated forest residues, which in turn inhibits hydrogenotrophic methanogens due to a drop in pH, demanding a higher amount of active microorganisms and/or enzyme activity [20, 21].

During continuous operation, an OLR of at most 4.4 g VS/L/day could be achieved. Around 90 % of the OLR came from forest residues where 45 % of VS is lignin. Since lignin is not digested in the biogas process, the OLR calculated from the remaining 55 % of the VS is 2.2 g VS/L/day. This is a more moderate OLR, and given the stable process shown in the present study, it points toward the possibility of using a higher OLR, while still maintaining stable conditions. A higher OLR would mean a better utilization of any given biogas plant. Furthermore, with an OLR of 2.2 g VS/L/day, the VS reduction will increase from 8 and 20 to 15 % (SD±6) and 33 % (SD±10) for digesters 1 and 2, respectively. Hence, NMMO treatment enables a doubling of the digestibility of forest residues. Nonetheless, there is still two thirds of the total digestible VS left in the digestate residue; thus, further work needs to be done to increase the VS reduction during the anaerobic digestion of forest residues. The lignin-rich digestate residue contains a high heating value and can be used as fuel for combustion in combined heat and power (CHP) plants [22]. The water content of the digestate should be decreased to 45 % TS prior to combustion [23].

In a previous study performed by Shafiei et al. [24], a techno-economic analysis for ethanol production from wood based on NMMO pretreatment was developed, and the process was designed to utilize 200,000 tons of spruce wood per year. The wastewater from this process with a large amount of unutilized pentoses was directed to an anaerobic digestion process for production of biogas. According to this study, the bioethanol production in combination with biogas production using NMMO-pretreated spruce as feedstock would be a feasible process. The total energy output in form of ethanol, lignin, and methane were calculated to be 134 MW/year and the share of the heat value generated from lignin residues is around 66 MW/year [24]. The total energy output based on the results in this present study can be calculated to about 85 MW/year, when 200,000 tons (dry weight) forest residues are utilized for biogas production in a continuous process. The energy output from lignin is about 80 MW/year, which is 21 % higher than for spruce 66 MW/year considering that spruce has higher carbohydrate content and lower lignin content than the forest residues used in this study. The production of biogas from lignocelluloses however would have a higher overall energy efficiency comparing to that for ethanol production; hence, pentoses can also be utilized in biogas production [25].

The methane yield obtained during the continuous process is approximately 60 % lower than the methane potential measured in the batch assay for both pretreated and untreated forest residues (Fig. 2 vs. Fig. 1). The lower yield could be due to the lower retention time of 20 days

used for the digestion of the substrate in the continuous process compared with 52 days digestion period in the batch process. The accumulated methane yields observed after 20 days of digestion time in the batch assay were about 25 Nml/g VS_{added} for untreated and 64 Nml/g VS_{added} for the pretreated material (Fig. 1). Comparing these data with the yield of biogas production of 53 and 92 NmL/gVS/day, during continuous digestion of untreated and treated forest residues, respectively (Fig. 2), and assuming 50 % methane in the produced biogas from carbohydrates [26], it can be concluded that the results from batch and continuous digestions are in accordance with each other. This also explains the relatively low VS reduction obtained.

Moreover, addition of carbon-rich materials, such as lignocelluloses, to digesters treating waste mixtures with low C/N ratios has previously shown to enhance the nutritional balance and stabilize sensitive processes [27]. It was also shown [28] that the material and the ratio, by which the forest residues are co-digested with, would have a significant effect for the economy of the process. Using organic fraction of municipal solid waste (OFMSW) for co-digestion instead of sludge and decreasing the ratio of forest residues in the mixture would increase the methane yield considerably, since OFMSW has higher methane potential than sludge.

Conclusion

Today, forest residues are available for energy production in Sweden on a scale of 59 TWh/year [3]. However, the use of this feedstock for biogas production is limited due to the lack of an efficient pretreatment enabling digestion of the cellulose and hemicellulose in the forest residue. The present study shows the possibility of one pretreatment method; however, an economic and technical assessment of its industrial use needs to be performed in the future. One aspect not evaluated in this study is the quality of the digestate. Since 45 % of the substrate is lignin that is not degradable, hence remains in the digestate residue, which would after de-watering have a potential value as a fuel for combustion.

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