Effect of the *N*-Methylmorpholine-*N*-Oxide (NMMO) Pretreatment on Anaerobic Digestion of Forest Residues

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Pretreatment of forest residues using *N*-methylmorpholine-*N*-oxide (NMMO or NMO) prior to anaerobic digestion was investigated, where the effects of particle size, NMMO concentration, and pretreatment time were the primary focus. The pretreatments were carried out on forest residues; with different particle sizes of 2, 4 and 8 mm, at 120 °C for 3, 7, and 15 h in two different modes of NMMO-treatment: dissolution by 85% NMMO and swelling without dissolution using 75% NMMO solution in water. The pretreatment process led to minor changes in the composition of the forest residues. The best improvement in methane yield of the forest residues was achieved by pretreatment using 85% NMMO for 15 h at 120 °C. This treatment resulted in 0.17 Nm^3/kg VS methane yield, which corresponds to 83% of the expected theoretical yield of carbohydrates present in the material. Additionally, the accumulated methane yield and the rate of the methane production were highly affected by the amounts of remaining NMMO when it was not well separated during the washing and filtration steps after the treatment. The presence of concentrations even as low as 0.008% NMMO resulted in a decrease in the final methane yield by 45%, while the presence of 1% of this solvent in the digester completely terminated the anaerobic digestion process.

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INTRODUCTION

Increased concern for the security of the oil supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has put pressure on societies to find renewable alternatives (Midilli *et al.* 2006). Bioenergy from renewable resources is a viable alternative to fossil fuels.

Among renewable energies, biogas has great potential as an alternative to fossil fuels. It can be utilized in the generation of power and heat, and it can also be upgraded to gaseous vehicle fuel (Börjesson and Mattiasson 2008; Klass 1998; Louwrier 1998; Saddler 1993). There are several studies that have been carried out on the conversion of wastes (*e.g.*, animal, industrial, household, and municipal) into biofuels by anaerobic biodegradation (Brown 2003; Cheng and Hu 2010; Elango *et al.* 2007; Forgács *et al.* 2012; Klass 1998). Large-scale biogas technologies utilizing a variety of wastes have already been developed in some countries in Europe, such as Germany, Sweden, and the Netherlands. However, to meet the increasing demand for bioenergy production, new raw

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materials have to be considered (Petersson *et al.* 2007). One of the most abundant wastes available for biofuel production is lignocellulosic biomass.

Lignocellulosic biomass refers to plant biomass, which is mainly composed of cellulose, hemicellulose, and lignin (Hendriks and Zeeman 2009; Malherbe and Cloete 2002; Percival Zhang *et al.* 2009) and represents the majority of renewable sources of potentially fermentable carbohydrates on earth (Nakamura and Mtui 2003).

However, the anaerobic digestion of lignocellulosic materials is limited by the rate of their hydrolysis (Boone 1984; Noike *et al.* 1985). The main biodegradable polymers in these kinds of biomass, cellulose and hemicellulose, are protected by lignin, a relatively inert three-dimensional polyphenylpropane polymer (Grohmann *et al.* 1986; Sarkanen and Ludwig 1971). This complex structure of lignocellulosic materials therefore results in physical and chemical barriers to biofuel production unless the structure is subjected to a suitable pretreatment prior to anaerobic digestion.

N-methylmorpholine-*N*-oxide (NMMO) is a cellulose solvent that is used industrially for the spinning of cellulosic fibers (the Lyocell process). Recently, it has been shown that when NMMO is used for pretreatment, there is a great improvement in biofuel production from lignocellulosic materials. NMMO is known to change the highly crystalline structure of cellulose after its dissolution and regeneration (Cuissinat and Navard 2006).

A few studies have been carried out on the optimization of NMMO pretreatment conditions prior to bioethanol and biogas production. Shafiei *et al.* (2010) performed NMMO pretreatment on spruce and oak prior to bioethanol production. The pretreatment of oak and spruce at 130 °C with 85% NMMO resulted in almost total conversion of cellulose into ethanol and improved the ethanol yields up to 85.4 and 89%, respectively. Poornejad *et al.* (2013) investigated the effects of NMMO-pretreatment on rice straw for bioethanol production. The results of their study showed a significant improvement in the enzymatic hydrolysis of rice straw followed by fermentation into bioethanol. These results showed a promising effect of NMMO pretreatment on enzymatic hydrolysis. However, because bacterial hydrolysis has a different mechanism than cellulase enzymatic hydrolysis, it is not possible to conclude that the NMMO pretreatment can also be conducive to biogas processes.

Regarding biogas production, Jeihanipour *et al.* (2009) studied NMMO pretreatment of highly crystalline pure cellulose, which resulted in subsequent 100% conversion of cellulose into methane after 15 days of digestion. In another study, Teghammar *et al.* (2012) investigated biogas production from rice and triticale straws and spruce chips by NMMO pretreatment. The best conditions for the NMMO pretreatment in their work led to 87% of the theoretical methane yield.

In this work utilization of forest residues for biogas production was investigated due to its abundance in Sweden. In 2008, the tree branches and tops that were received from Swedish forests amounted to about 1.6 megatonnes total solids/year. This amount is expected to increase to 3.5 megatonnes total solids/year by 2018 (Thuresson 2010).

This paper addresses two main issues regarding NMMO pretreatment prior to biogas production: first, the optimization of the NMMO pretreatment was performed on an inhomogeneous waste stream of lignocellulosic biomass, *i.e.,* forest residues. The pretreatments were carried out in two modes of action, *i.e.* dissolution and swelling using 85% and 75% NMMO, respectively. Furthermore, the effect of particle size (2, 4, and 8 mm) and the treatment time (between 3 and 15 h) were also considered. Secondly, since, traces of remaining NMMO from the pretreatment may affect the anaerobic digestion

process, the presence of different concentrations of NMMO in anaerobic digestion process were studied. This is the first work on NMMO pretreatment of forest residues and the limitations caused by NMMO in anaerobic digestion systems.

EXPERIMENTAL

Materials

Native forest residue, an inhomogeneous mixture of spruce, pine bark, *etc*., was obtained from the forest outside Borås, Sweden. The material was dried at room temperature for a couple of days and then cut, milled, and screened to achieve three different fractions with particle sizes of 2, 4, and 8 mm.

Methods

Industrial-grade (50% w/w) NMMO solution (BASF, Ludwigshafen, Germany) was used in all pretreatment experiments. The concentration of NMMO was first increased to 75% and 85% (w/w) using a rotary evaporator (Laborata 20 eco, Heidolph, Germany) operating at an absolute pressure of 100 mbar and a maximum temperature of 130 °C. The NMMO solution was supplemented with 0.625 g/kg propylgallate to prevent oxidation of the NMMO during pretreatment (Bang *et al*. 1999; Kim *et al*. 2006).

For the pretreatments, 94 g of 85% or 75% NMMO solution were mixed with 6 g dry weight of forest residues with particle sizes of 2, 4, or 8 mm in 250-mL blue-cap bottles (Lennartsson *et al*. 2011). The bottles were then placed in an oil bath at 120 °C for 3, 7, and 15 h. The mixtures were stirred every 15 min with a glass rod (Shafiei *et al*. 2010), except for the 15-h pretreatment, where the mixtures were left overnight without mixing after 7 h. The pretreatment was stopped, and the cellulose was recovered by the addition of 150 mL of boiled distilled water followed by vacuum filtration and washing with hot (40 to 50 ºC) distilled water until a clear filtrate was achieved (Shafiei *et al*. 2010). The pretreated materials were stored at 4 °C until further investigations were conducted in anaerobic digestion assays. In addition, part of the materials was freeze-dried to prepare samples for further analyses.

Batch Anaerobic Digestion Assays

Batch digestion assays were carried out according to the method described by Hansen *et al.* (2004) using thermophilic inoculum obtained from a large-scale digester treating municipal solid waste at 55 °C (Borås Energy and Environment AB, Sweden). The total solids (TS), volatile solid (VS), and volatile fatty acids (VFA) content of the inoculum was 2.77 %, 1.68 %, and 1.90 %, respectively. The digesters used in the assays were serum glass bottles with 118 mL of total volume that were closed with butyl rubber seals and aluminum caps. Each flask contained 30 mL of inoculum and 0.25 g volatile solids (VS) of substrate to achieve a VS ratio of inoculums to substrate of 2:1. Furthermore, inoculums alone were used as blanks for the determination of the gas production of the inoculum itself. In addition, pure cellulose (Cellulose Fibrous Long, Sigma Aldrich, Germany) was used as a control substrate to check the quality of the inoculum. Moreover, the inhibition effect of NMMO was investigated by digestion of pure cellulose fibers in the presence of different concentrations (between 6.4×10^{-5} and 1%) of NMMO.

All experimental setups were performed in triplicate. Finally, the headspace of each bottle was flushed with a gas mixture of 80% nitrogen and 20% carbon dioxide to obtain anaerobic conditions. Gas samples were withdrawn regularly from the headspace of each bottle and analyzed by gas chromatography (GC) to obtain the accumulated methane production during the digestion period of 50 days. The amount of methane produced in the reactor headspace was then calculated using the data from the GC measurements as described by (Teghammar *et al*. 2010).

Analytical Methods

The total solids (TS) and volatile solids (VS) in the different samples were determined by first oven drying to a constant weight at 105 °C , followed by ignition at 575 °C in a furnace (Sluiter *et al*. 2008a). The cellulose, hemicellulose, and lignin contents of the pretreated or untreated lignocelluloses were determined according to NREL procedures (Sluiter *et al*. 2008b). In this method, a two-step acid hydrolysis with concentrated and diluted sulfuric acid was performed to liberate the sugars from the cellulose and the hemicellulose. The formed sugars were then quantified by HPLC. The acid-soluble lignin was measured using UV spectroscopy at 280 nm, and acid-insoluble lignin was determined after drying followed by ignition at 575 °C. All lignin and carbohydrate analyses were performed in duplicate.

The total carbohydrate (cellulose and hemicelluloses) were analyzed using 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 the eluent with a flow rate of 0.6 mL/min.

The methane produced in anaerobic digestion was measured using a gas chromatograph (Auto System PerkinElmer, Inc., Waltham, MA) equipped with a packed column (PerkinElmer, 60x1, 800OD, 80/100, Mesh) and a thermal conductivity detector (PerkinElmer) with an injection temperature of 150 °C. The carrier gas used was nitrogen, with a flow rate of 23 mL/min at 60 °C. A 250-µL pressure-tight gas syringe (VICI, Precision Sampling Inc., LA) was used for the gas sampling. Excess gas was released through a needle after the gas analyses to avoid overpressure higher than 2 bar in the head space of the flasks. All methane volumes are presented at standard condition (temperature 273 K, and pressure 101,325 Pa).

Kinetic Model

A first-order kinetics model described previously by Jimenéz *et al.* (2004) was used to determine the inhibition effects of the presence of different concentrations of NMMO on the anaerobic digestion process,

$$G = G_m(1 - e^{-K_0 t}) , \qquad\qquad (1)$$

where G is the accumulated methane volume (mL) after a time t (days), G_m is the maximum accumulated methane volume (mL) after an infinite digestion time, and K_0 is the observed specific rate constant of the overall process (days^{-1}). To calculate the value of the specific rate constant, Eq. (1) is transformed as follows:

$$Ln(\frac{G_m}{G_m - G}) = K_0 t \qquad (2)$$

Statistical Analysis

All experiments in this study were carried out in triplicates. The significant differences between methane productions obtained by anaerobic batch digestion assays of untreated *vs* treated samples was verified by t-tests using a software package MINITAB® (V 15.0). All error bars and intervals reported represent 95% confidence intervals.

RESULTS AND DISCUSSION

Pretreatment of forest residues with particle sizes of 2, 4, and 8 mm, was performed using 75 and 85% w/w NMMO solution at 120 °C for 3, 7, and 15 h, and the effects of the pretreatment on the composition and the methane yield were investigated. This organic solvent has shown a high potential to enhance the digestibility of lignocellulose. However, so far little attention has been paid to possible inhibitory effects of this solvent in an anaerobic digestion system. Therefore, the effects of different concentrations of NMMO in the anaerobic digestion process were also explored in this study. The purpose of this investigation was to verify that the presence of the solvent after insufficient washing following the pretreatment step might inhibit the anaerobic digestion process.

Carbohydrate Composition of Untreated and NMMO-Treated Forest Residues

The results of the compositional analyses regarding the contents of total carbohydrates and total lignin were carried out only on the smallest particle size (2 mm) of the forest residues (Table 1). Other components, such as extractives and acetyl content, were not analyzed. The content of total carbohydrates in the untreated forest residues was 41.6 %. The content of total carbohydrates increased slightly as a result of the NMMO treatment, achieving values between 44.1 and 49.3 % (Table 1). The highest total carbohydrate content was obtained when the longest treatment time (15 h) and 85% NMMO was applied. While the content of total carbohydrates increased with increased treatment times, the total lignin content decreased. The total lignin content (acid soluble lignin and acid insoluble lignin) of untreated forest residues was 43.4 %, and this value was reduced after the treatment to between 37.4 and 39.2 % (Table 1). In general, the results of the compositional analyses show that the treatment did not seriously affect the composition of the substrate. These results are in accordance with previous findings of NMMO pretreatment of spruce, birch, and rice straw (Goshadrou *et al*. 2013; Poornejad *et al*. 2013; Teghammar *et al*. 2012).

Effects of NMMO-Pretreatment on Anaerobic Digestion

The results of accumulated methane yields obtained after 50 days of digestion are shown in Fig. 1. The methane potential of untreated assays of forest residues with particle sizes of 2, 4, and 8 mm were 0.07 ± 0.007, 0.031 ± 0.009, and 0.00 Nm^3 CH$_4$/kgVS, respectively. However, after the pretreatment, methane yields increased up to 10, 15, and 50 times for particle sizes of 2, 4, and 8 mm, respectively.

Table 1. Pretreatment Conditions, Lignin and Carbohydrate Content, Initial Methane Production Rates, and Accumulated Methane Yields of Untreated and Treated Forest Residues

NMMO Conc. (%)	Time (h)	Total Carbohydrates (%)	Total Lignin (wt %)	Initial methane production rates * (Nm^3CH_4/kg VS)	Accumulated methane yield (Nm^3CH_4/kg VS)
Untreated	-	41.6	43.4	0.005	0.07± 0.007
85%	15	49.3	38.1	0.012	0.17± 0.020
85%	7	45.3	39.6	0.009	0.15± 0.018
85%	3	45.2	40.6	0.003	0.12± 0.028
75%	15	46.2	37.4	0.004	0.11± 0.014
75%	7	46.0	38.4	0.003	0.11± 0.012
75%	3	44.1	39.2	0.000	0.13± 0.051

* Initial digestion rate determined as the methane production per day during the first 12 days of the digestion period

The best results of anaerobic digestion were obtained when forest residues with 2-mm particle size were treated with 85% NMMO for 15 h, resulting in a methane yield of 0.17 ± 0.020 Nm^3 CH_4/kgVS. This is an improvement by 152% compared with the yield of 0.07 ± 0.007 Nm^3 CH_4/kgVS measured from untreated forest residues (Fig. 1A). The decrease in NMMO concentration to 75% contributed to a lower methane yield of 0.13 ± 0.051 Nm^3 CH_4/kgVS after 3 h of treatment. In contrast, longer pretreatment time did not necessarily lead to a higher biogas yield (Fig. 1A). Treatment of forest residues with larger particle sizes of 4 and 8 mm resulted in methane yields of up to 0.10 and 0.05 Nm^3 CH_4/kgVS, respectively. This was to be expected because decreasing the particle size increases the surface area, which will in turn lead to better enzymatic degradation (Taherzadeh and Karimi 2008; Teghammar *et al*. 2012).

To verify the significance of differences between the methane yields obtained from untreated *vs.* treated samples, a statistical analysis using *t-test* was performed on data showing the best performance *i.e.* 2 mm particle size. The results showed that the enhancement in the accumulated methane production after the treatment was significant when pretreatment time of 15 h was applied in both dissolution (85% NMMO) and swelling (75% NMMO) mode (p-value 0.001 and 0.005, respectively) (Fig. 1A and Table 2). Whereas, applying pretreatment time of 7 h, showed significant effect only in the case of dissolution mode (85% NMMO), p-value 0.003 (Fig. 1A and Table 2). The 3-h pretreatment did not cause any significant increase in the accumulated methane production in any cases.

Table 2. Evaluation of significant differences between the accumulated methane yields of untreated *vs.* pretreated samples of 2mm particle size

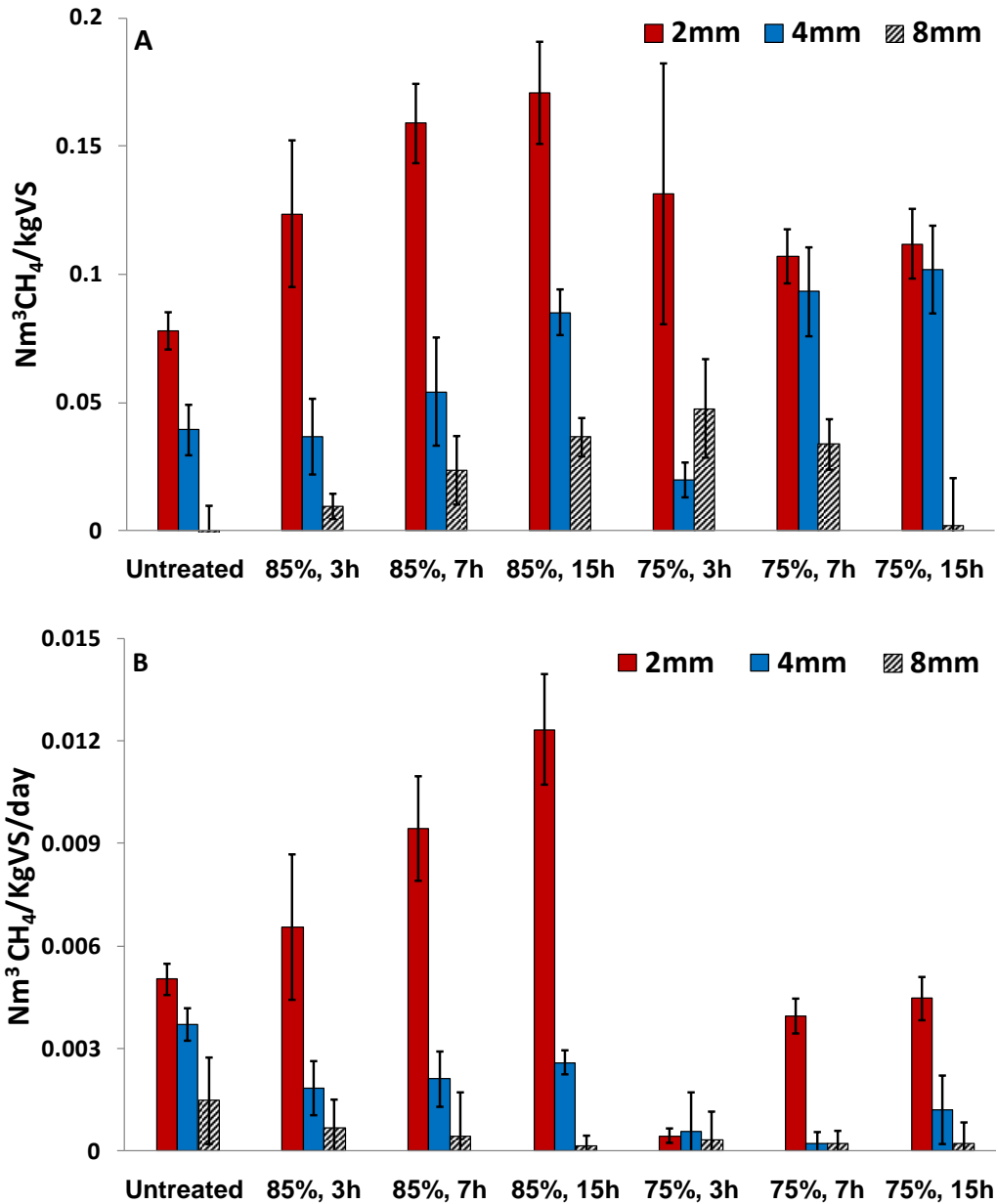| NMMO Conc. (%)	Time (h)	P-Value Compared to Untreated Sample	Significant Difference Yes/No
85%	3	0.059	No
85%	7	0.003	Yes
85%	15	0.001	Yes
75%	3	0.240	No
75%	7	0.061	No
75%	15	0.005	Yes

Fig. 1. Accumulated methane yield during 50 days of anaerobic digestion of untreated and NMMO-pretreated forest residues expressed as Nm³ CH₄/kgVS (A) Initial digestion rate determined as the mean of the methane production per day during the first 12 days of the digestion period expressed as Nm³ CH₄/kgVS/day (B).

These results indicate that changes in the structure of highly crystalline cellulose in cellulose dissolution and swelling without dissolution are completely different processes (Jeihanipour *et al*. 2009; Zhang *et al*. 2006). The dissolution mode of NMMO pretreatment was more successful because the hydrogen bonds and weak van der Waals forces between cellulose chain molecules break in this mode of action. Once these forces are broken during the dissolution, the chains are free to rearrange themselves. Subsequently, when NMMO is removed, the cellulose chains can create new bonds in a less crystalline state. However, in cellulose swelling mode, the gross structure of the

cellulose remains unchanged, even though significant physical changes resulting in an increase in the sample volume by uptake of the NMMO take place (Zhao *et al*. 2007).

The initial reaction rates were determined as the means of the methane production per day during the first 12 days of the incubation period and are presented in Fig. 1B. Again, the highest digestion rate of 0.012 Nm^3 CH_4/kgVS/day was achieved when forest residues with 2-mm particle size were treated with a higher concentration of NMMO (85% w/w) for 15 h.

For larger particle sizes (*i.e*., 4 and 8 mm), however, a long lag phase was observed (data not shown). This might be due to the low efficiency of the NMMO pretreatment on larger particle sizes for reducing the highly crystalline cellulose. Weimer *et al.* (1990) reported that the presence of highly crystalline cellulose in digestion may lead to much longer lag time compared to amorphous cellulose. Their explanation for this phenomenon was that the cellulolytic microorganism may attach more rapidly to and/or more readily recognize the amorphous cellulose than the crystalline cellulose (Weimer *et al*. 1990, 1991.

Additionally, comparisons between the initial reaction rates of the pretreated assays with particle sizes of 4 and 8 mm and untreated assays with similar particle sizes showed noticeably slower reaction rates (Fig. 1B). This might be due to the inhibitory effect of the remaining NMMO on the anaerobic digestion process. This finding is in accordance with previous work on oil palm empty fruit bunch (OPEFB), where it was found that the presence of commercial NMMO can significantly inhibit the process of digestion (Purwandari *et al*. 2013). In another study, the inhibitory effect of NMMO on Zygomycetes fungi was also observed during bioethanol production (Lennartsson *et al*. 2011). However, as shown in Fig. 1A, the accumulated methane production of the pretreated materials with larger particle sizes was higher compared to that of the untreated ones, which shows that the methanogen bacteria may adapt to the presence of small amounts of NMMO that is eventually present in the broth during the longer period of the digestion tests.

In general, pretreatment with NMMO is a beneficial method compared to many other pretreatments because the composition of the treated wood remains unchanged, including the hemicelluloses (Purwandari *et al*. 2013; Shafiei *et al*. 2010). Furthermore, it provides high flexibility in the choice of lignocellulosic feedstocks (Rosenau *et al*. 2001). However, the main drawbacks of NMMO pretreatment are longer pretreatment times and the need for a very efficient recovery and recycling of the treatment chemical after the treatment (Hall *et al*. 1999).

NMMO as an organic solvent possesses a highly polar nature that provides an excellent disruption of the extensive hydrogen-bonded network formed by carbohydrate polymers (Kuo and Lee 2009; Rosenau *et al*. 2001). The water added at the end of the treatment acts as an anti-solvent agent, leading to the regeneration of cellulose. During this dissolution regeneration process, the crystalline structure of cellulose I changes into cellulose II, making it more accessible to the degrading cellulolytic enzymes during the anaerobic digestion.

The results of this work shows that the interaction between the solvent and the forest residues seems to be more effective when decreasing the particle size and increasing the treatment time (Fig. 1). Additionally, increasing the concentration of the solvent (from 75% to 85%) showed considerable improvement in digestibility. This result is in agreement with Jeihanipour *et al.* (2009), who reported an efficient conversion of

cellulose I into cellulose II by treating cellulose fibers in 85% NMMO prior to enzymatic hydrolysis.

Inhibition Effects of NMMO on the following Anaerobic Digestion Process

Despite the positive effects of NMMO pretreatments, one of the drawbacks might be the presence of the solvent after insufficient washing, which might inhibit the subsequent anaerobic digestion process. Purwandari *et al.* (2013) examined the inhibitory effect of the NMMO in the batch mode of anaerobic digestion. For this purpose, 2.5 g/L commercial NMMO solution was added to the inoculum and digested at 55 °C. The results of their study showed that only 15% of the expected gas production from the inoculum was achieved in the presence of the NMMO at this concentration. For that reason, in this work, a more detailed analysis of the inhibitory effects has been carried out. Anaerobic digestion assays on pure cellulose with NMMO added at different concentrations (between 0 and 1%) were performed. All the reactors contained 8 g VS/L cellulose, and the results of the accumulated methane production during the 50-d incubation period are shown in Fig. 2A. The results indicate that NMMO concentrations as low as 0.0016% can reduce the accumulated methane yield by 34% (Fig. 2A and Table 3). No inhibition has been observed at concentrations below 0.000064%. However, the methane yield was decreased by almost 50% in reactors containing NMMO at concentrations between 0.0016 and 0.02%. Moreover, the highest concentration of NMMO (1%) resulted in negligible methane yield, indicating that the microorganisms involved in the digestion process were completely inhibited.

Previously, Jeihanipour *et al.* (2009), examined the effect of addition of 0.5% NMMO on enzymatic hydrolysis of cellulose, which reduced the hydrolysis rate by 12 %. In contrast in this work, 51% reduction in accumulated methane production from cellulose was obtained after addition of 0.2% NMMO in the anaerobic digestion system. This reveals a high adverse sensitivity of the methane-producing microorganism to this organic solvent. Additionally, it explains that the mechanism of the methane-producing microorganisms is rather different from the enzymatic hydrolysis.

The degradation pathway of NMMO begins with the reduction of NMMO to N-methylmorpholine (NMM), which is subsequently demethylated and transformed into morpholine and formaldehyde (Rosenau *et al*. 2001). NMMO was considered to be persistent until Meister and Wechsler (1998) showed that it could be metabolized by certain microbial species/environments as activated sludge, anaerobic degradation processes, and two yeast cultures (Fig. 3).

The adaptation of the microorganisms to NMMO and its metabolites is a sequential process. First, the microorganism must be adapted to NMMO to form NMM. The adaptation to NMM can take a number of days to reach a certain threshold concentration. Therefore, the NMM degradation cannot start until NMMO has been reduced to NMM. In the same way, morpholine degradation is only possible until the sludge is adapted to NMM. Morpholine is thus a much better biodegradable compound than NMMO or NMM (Schräder *et al*. 2000).

The reduction of NMMO to NMM was also observed under anaerobic conditions; however, the reaction stopped at NMM, and no further biodegradation was obtained, even with the presence of a co-substrate such as glucose, under the conditions tested (Knapp *et al*. 1996).
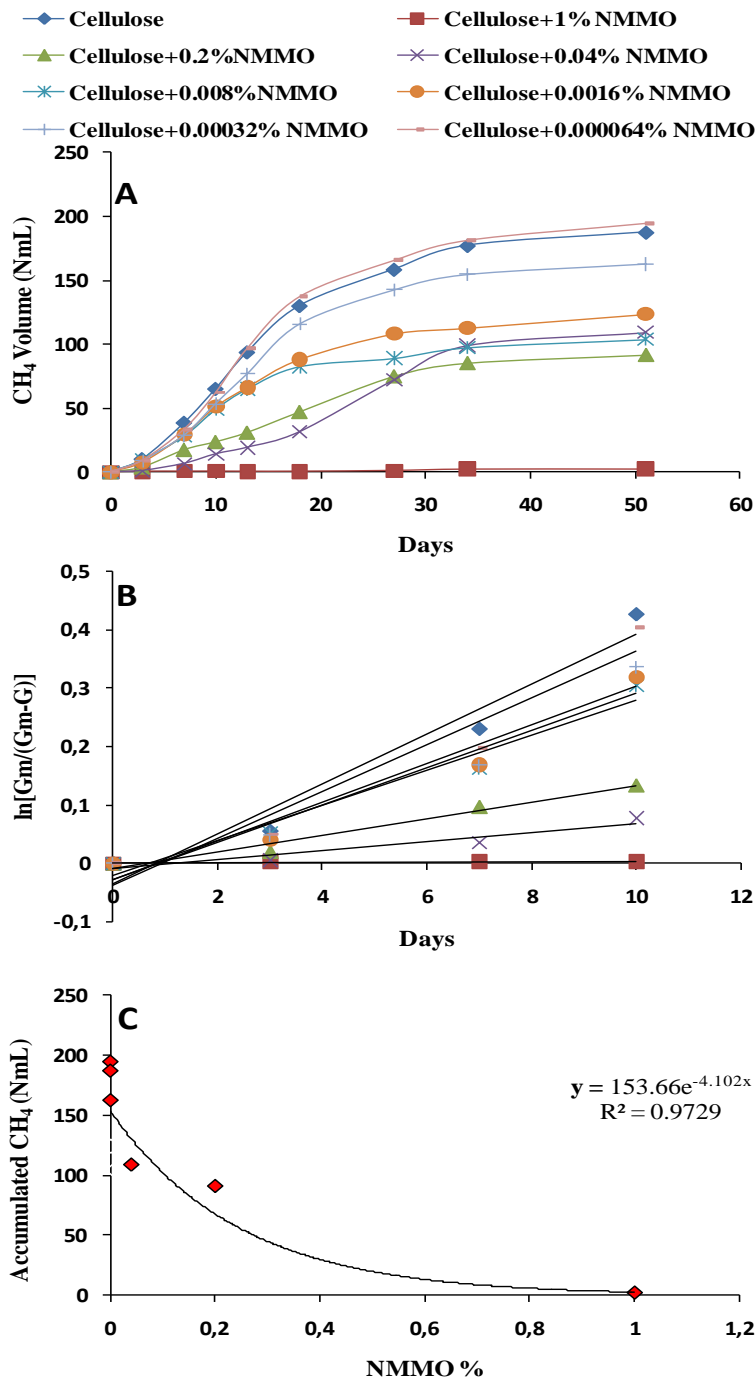
Fig. 2. Methane production obtained from cellulose with the addition of different concentrations (0.000064 to 1%) of NMMO. Accumulated produced volume CH4 (mL) during the incubation period of 50 days (A) Kinetic evaluation of the digestion process: values of ln[Gm/(Gm-G)] as a function of time (days) for pure cellulose and cellulose together with different concentrations (0.000064 to 1%) of NMMO (B) Correlation between accumulated methane yield (NmL) and NMMO concentrations (C)
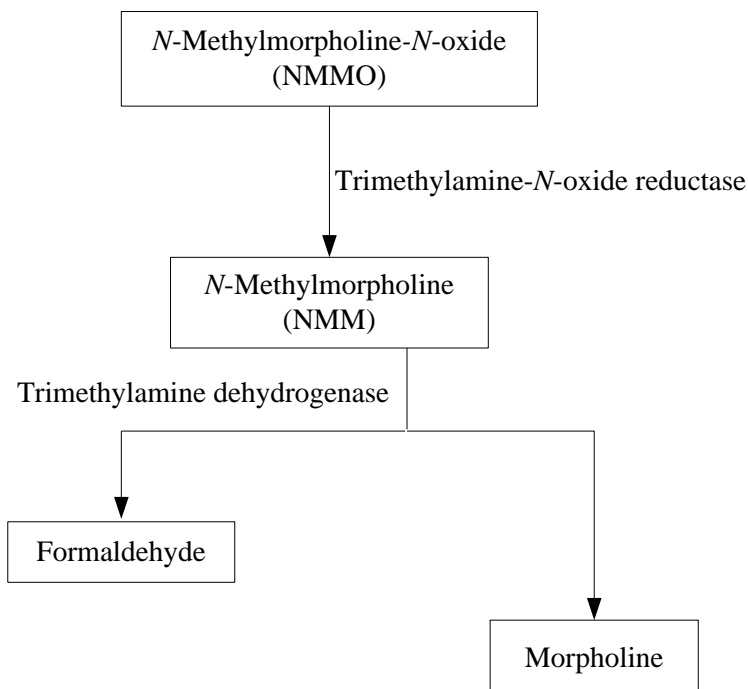
Fig. 3. Main degradation products of NMMO (Meier and Turnbull 2013)

To characterize the inhibition effects, a first-order kinetics model was used (Jiménez *et al*. 2004). Figure 2B provides information about the kinetics of the degradation within the first 10 days of digestion. The results show that not only accumulated methane production (Fig. 2A), but also the degradation rate declined with increasing NMMO concentrations in the reactors (Fig. 2B and Table 3). The methane production rate and NMMO concentration in the digester were correlated ($R^2=0.973$ in Fig. 2C). Moreover, the results presented in Table 3 show a direct correspondence between the NMMO concentrations and final methane yield in the systems.

Table 3. Accumulated Methane Production and Specific Rate Constant K_0 Obtained During 50 Days of Incubation of Cellulose with Different Concentrations of NMMO

Sample sets	Specific rate constant K_0 (day^{-1})	Accumulated methane production (NmL)	Final methane yield compared to pure cellulose (%)
Cellulose +1% NMMO	0.0003	2.42	1.30
Cellulose +0.2% NMMO	0.0142	91.30	48.71
Cellulose +0.04% NMMO	0.0077	109.15	58.23
Cellulose +0.008% NMMO	0.03	103.95	55.46
Cellulose +0.0016% NMMO	0.0319	123.63	66.00
Cellulose +0.00032% NMMO	0.0331	162.85	86.88
Cellulose +0.000064% NMMO	0.0399	194.99	104.05
Cellulose	0.0428	187.43	–

*The inhibition effects are expressed as percentage of methane yield of that obtained for the control, *i.e.,* pure cellulose fibers.

The values of K_0 obtained for cellulose with no addition of NMMO and in the presence of very low concentrations of NMMO (0.000064% and 0.00032%) was 0.04, 0.04, and 0.03 d^{-1}, respectively, which was considerably decreased in the presence of higher concentrations of NMMO (Table 3). Finally, the results of this study showed that NMMO could have a significant effect on anaerobic digestion. However, because no NMMO levels were measured throughout the digestion process, it is not possible to establish if NMMO was reduced to NMM and whether it was the NMM accumulation or the NMMO itself that was the factor resulting in the inhibition of the process. Further investigations are therefore recommended to study the degradability of NMMO and its metabolites in anaerobic systems.

CONCLUSIONS

1. The dissolution mode of NMMO treatment using 85% NMMO resulted in 83% of the theoretical yield, which is almost three-fold higher methane production compared to that observed from untreated forest residues.

2. The advantage of NMMO pretreatment is that it does not cause destruction of cellulose and hemicellulose, while the lignin content was decreased by approximately 7% when the longest pretreatment time (15 h) was applied.

3. The washing and filtering steps seem to be critical for the performance of the subsequent anaerobic digestion process, as NMMO remaining in concentrations higher than 0.002% considerably decreased the methane yield.

ACKNOWLEDGMENTS

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