PRETREATMENT OF STRAW FRACTION OF MANURE FOR IMPROVED BIOGAS PRODUCTION

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Pretreatment of straw separated from cattle and horse manure using N-methylmorpholine oxide (NMMO) was investigated. The pretreatment conditions were for 5 h and 15 h at 120 °C, and the effects were evaluated by batch digestion assays. Untreated cattle and horse manure, both mixed with straw, resulted in 0.250 and 0.279 Nm^3 CH_4/kgVS (volatile solids), respectively. Pretreatment with NMMO improved both the methane yield and the degradation rate of these substrates, and the effects were further amplified with more pretreatment time. Pretreatment for 15 h resulted in an increase of methane yield by 53% and 51% for cattle and horse manure, respectively. The specific rate constant, k_0, was increased from 0.041 to 0.072 (d^{-1}) for the cattle and from 0.071 to 0.086 (d^{-1}) for the horse manure. Analysis of the pretreated straw shows that the structural lignin content decreased by approximately 10% for both samples and the carbohydrate content increased by 13% for the straw separated from the cattle and by 9% for that separated from the horse manure. The crystallinity of straw samples analyzed by FTIR show a decrease with increased time of NMMO pretreatment.

Keywords: Anaerobic digestion; Manure; Straw; Pretreatment; N-Methylmorpholine Oxide

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INTRODUCTION

A reliance on fossil fuels as the main energy source has caused several environmental and economical challenges (Budiyono et al. 2010). Thus, there is a steadily rising worldwide interest in investigating renewable sources for energy production (Amon et al. 2007). Anaerobic digestion (AD) is a technology generally used for management of organic waste for biogas production, since it offers a renewable source of energy and at the same time solves ecological and agrochemical problems (Budiyono et al. 2010). A variety of raw materials, among others energy crops and animal manure, can be utilized as organic matter for biogas production (Neves et al. 2009).

Methane is produced during the anaerobic degradation of the organic components such as carbohydrates, proteins, and lipids present in the manure. The ultimate methane yield is affected by several factors, such as the feed, species, breed, and growth stage of the animals as well as the amount and type of the bedding material, together with the pre-storage conditions prior to biogas production (Møller et al. 2004). The composition, i.e., the protein, fat, fiber, cellulose, hemicellulose, starch, and sugar content, are also important factors that influence the methane yield (Comino et al. 2009).

Straw, when used as bedding material in proper ratios or after appropriate pre-treatment, can beneficially affect the methane yield by enabling a more advantageous carbon to nitrogen (C/N) ratio for the substrate (Hashimoto 1983). Since straw belongs to the class of difficult-to-degrade lignocellulosic materials, a pretreatment step is needed to improve the rate and degree of enzymatic hydrolysis during the degradation process. Lignocelluloses are composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. The cellulose and hemicellulose are sheltered by lignin, which provides integrity and structural rigidity. The content and distribution of lignin is responsible for the restricted enzymatic degradation of lignocelluloses, by limiting the accessibility of enzymes (Taherzadeh and Karimi 2008). Therefore, to improve biogas formation, often an effective and economically feasible pretreatment step is necessary. However, most of the reported methods such as dilute acid, hot water, AFEX, ammonia recycle percolation, and lime treatments are costly and have strong negative environmental effects, while others such as biological pretreatments are time consuming (Taherzadeh and Karimi 2008).

A study on the efficiency of biogas production of plant residues in co-digestion with cattle manure (Hassan Dar and Tandon 1987) showed that pretreatment of plant residues resulted in increased biogas yield by 31 to 42%. On the other hand, the rate of the bioconversion was very slow. They have also reported that caustic soda pretreatment has a promising effect on the delignification process compared to other pretreatments using sulphuric acid, phosphoric acid, ammonia, sodium hypochlorate, and acetic acid. However chemical pretreatments also can have strong negative environmental effects.

N-Methylmorpholine-*N*-oxide (NMMO) is one of the non-derivatizing solvents that can break the intermolecular interactions in cellulose, and it is mainly used in the textile industry for spinning of cellulose fibers (Lyocell process). It is considered to be environmentally friendly, since it does not generate toxic pollutants and it is recyclable with more than 98% recovery. Furthermore, NMMO is known to modify the highly crystalline structure of cellulose, while leaving the composition of wood intact and causing no hydrolysis of the hemicellulose (Lennartsson et al. 2011). Another study (Shafiei et al. 2009) showed that NMMO pretreatment of oak and spruce resulted in an increase of the digestibility during a following enzymatic hydrolysis.

The objective of this study was to investigate the effects of NMMO pretreatment on biogas production from horse and cattle manures, both with a high content of straw. The manure samples were first separated to obtain the straw fraction for the NMMO pretreatment. The pretreated fractions were then mixed back with the rest of the samples, and the biogas production was determined and compared with that of the untreated samples using anaerobic batch digestion tests.

MATERIAL AND METHODS

Materials

Two different deep litter manures obtained from a horse farm and a cattle farm outside Borås (Sweden) were investigated. The characterization of the substrates was carried out by Analys- & Konsulatlaboratoreiet Borås, Sweden, according to standard methods and the data are summarized in Table 1.

Table 1. Characterization of the Horse and Cattle Manures Mixed with Straw

Analyses	Horse manure	Cattle manure
Total Solids(wt%)	81.5	23.0
Volatile Solids(wt%)	61.8	18.1
Protein(wt%)	11.0	4.80
Kjeldahl Nitrogen(wt%)	1.70	0.76
Ammonium Nitrogen(wt%)	0.047	0.017
Fat Content(wt%)	1.60	0.30
Carbohydrates(wt%)	49.2	13.0

Pretreatment Procedure

The straw fraction of the manure was separated for each pretreatment condition according to a procedure shown in Fig. 1, where 7.5 g of manure was washed with 150 mL hot tap water and then filtered using a coarse vacuum filter with 1 mm pore size. The filtrate was collected and stored at -20ºC until further utilization.

The pretreatment of each straw fraction was carried out using a commercial grade NMMO (50% w/w in aqueous solution) solution (BASF, Ludwig-Shafen, Germany). In order to achieve a concentration of 85%, the NMMO solution was evaporated in a vacuum evaporator. Then, propylgallate was added to a concentration of 0.6 g/L. It is an antioxidant and prohibits oxidation and deterioration of the solvent during the following pretreatment procedure (Shafiei et al. 2009). Each straw fraction was then pretreated with 92.5 g of 85% NMMO solvent in an oil bath at 120ºC for 5 h or 15 h. Under the 5 h pretreatment, the suspension was mixed every 15 min, while during the 15 h pretreatment, the suspension was left overnight without mixing. After the pretreatments, the NMMO was separated by adding boiling tap water and then filtered through a coarse vacuum filter. This washing and filtering step was repeated a few times, until the filtrate was clear, indicating that the NMMO solvent was completely washed out. The filtrate was then centrifuged (5 min, 5000 rpm) in order to obtain fine particles, which had passed through the filter, and the supernatant was discarded. The pellet was also repeatedly washed with hot boiling water and centrifuged until the supernatant was clear and the NMMO solvent was completely washed out. The pretreated straw together with the fine particles were dried in a freeze dryer and kept at 4ºC until use.

Anaerobic Batch Digestions

The anaerobic batch digestion experiments were carried out according to a previously published method (Hansen et al. 2004). The digesters used were 118 mL glass bottles closed with a rubber septum and aluminum caps. The inoculum was obtained from a 3000-m^3 municipal solid waste digester operating under thermophilic conditions (Borås Energi och Miljö AB, Sweden), and was incubated and stabilized at 55ºC for three days before use.

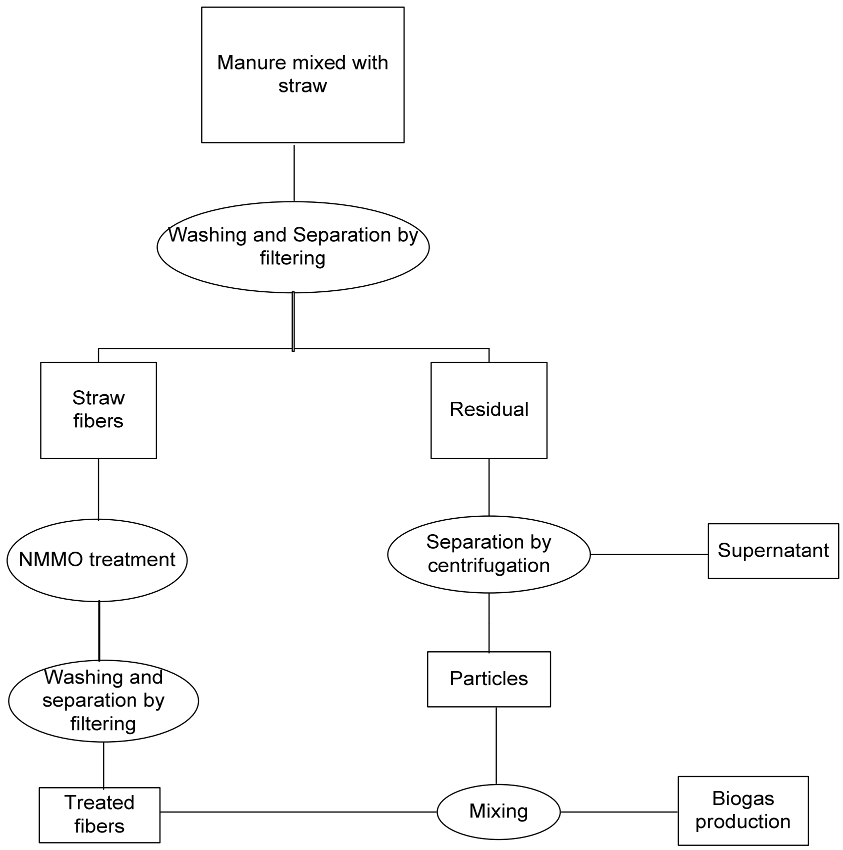

Fig. 1. Schematic presentation of the pretreatment process

The filtrate collected after the separation of straw was centrifuged at 5000 rpm for 5 min. The supernatant was discarded, and the sediment was mixed with the NMMO-pretreated straw fraction and used as substrate for the biogas production. Each reactor contained 40 mL inoculum, 0.3 g volatile solid of pretreated or untreated manure straws, and tap water to bring the total volume to 45 mL. In order to determine the methane production from the inoculum itself, blanks containing only inoculum and tap water were also examined in order to determine the biogas production from the substrate. In order to facilitate anaerobic conditions and to prevent pH-change, the head space of each reactor was finally flushed with a gas mixture of 80% N_2 and 20% CO_2. All experimental set-ups were performed in triplicates, and the reactors were then incubated at 55ºC for 52 days. During this experimental period, the reactors were shaken once per day.

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

Kinetic Modeling

The kinetics of the degradation process were evaluated using the following first-order kinetic model (Jiménez et al. 2004):

$$G = G_m\,[1-\exp(-k_0 t)] \quad (1)$$

or

$$\ln[G_m / (G_m - G)] = k_0 t \quad (2)$$

where G (mL) is the volume of methane accumulated after a period of time t (days), G_m (mL) is the maximum accumulated gas volume at an infinite digestion time, k_0 (day^{-1}) is the specific rate constant, and t (days) is the digestion time. Plotting the calculated data of $\ln[G_m/(G_m-G)]$ *vs.* time, t, gives a straight line with a slope equal to k_0 with intercept of zero. The value of G_m was considered equivalent to the volume of accumulated methane at the end of the experiments.

Analytical Methods

The total solids (TS) and volatile solids (VS) were determined according to Sluiter at al. (2005). Kjeldahl nitrogen and protein content were determined according to Swedish standard method ISO 25663 (Swedish Standard Institute, 1984), in which the materials are treated with a strong acid in order to release nitrogen, which can be then determined by titration. Since the Kjeldahl method does not measure the protein content, an average conversion factor of 6.4 is used to convert the measured nitrogen concentration to a protein concentration. For determination of ammonium nitrogen, the SIS 028134-1 method (Swedish Standard Institute 1976) was used. It is based on sparging the samples with deionized water and mixed it with ammonium citrate and reagents containing sodium nitroprusside, phenol, and sodium hypochlorite before analysis. Fat content was determined according to Method no. 131 (Nordic Committee on Food Analysis 1989). The method is based on treatment with hot concentrated hydro-chloric acid to release fat bound to protein, prior to extraction of the fat with diethylether. The structural carbohydrates and lignin content of the pretreated and untreated straw fractions were determined using a two-step hydrolysis method that has been used for lignocelluloses (Sluiter et al. 2008). The acid-soluble lignin was measured using a UV spectrophotometer, while acid-insoluble lignin was determined after ignition of the samples at 575ºC. The quantification of the sugars formed was performed by HPLC (Waters 2695, Millipore and Milford, USA) equipped with a refractive index (RI) detector (Waters 2414, Millipore and Milford, USA), using a Pb-based ion exchange column (Aminex HPX-87P, Bio-Rad, USA) with 0.6 mL/min pure water at 85°C, or a H-based ion exchange column (Aminex HPX-87H, Bio-Rad) at 60°C with 0.6 mL/min 5 mM H_2SO_4 as eluents.

The NMMO-pretreated straws were analyzed using a Fourier transform infrared (FTIR) spectrometer (Impact, 410, Nicolet Instrument Corp., Madison, WI). The spectra were achieved with an average of 32 scans and a resolution of 4 cm^{-1} in the range from 600 to 4000 cm^{-1} and controlled by Nicolet OMNIC 4.1 analyzing software (Jeihanipour, et al., 2009). The methane and carbon dioxide analyses were carried out using a gas chromatograph (Auto System, Perkin Elmer, USA) equipped with a packed column (Perkin Elmer, 6' x 1.8" OD, 80/100, Mesh, USA) and a thermal conductivity detector (Perkin Elmer) with inject temperature of 150°C. Nitrogen was used as carrier gas at 75ºC with a flow rate of 20 mL/min. For gas sampling, a 250µL pressure-tight syringe (VICI, Precision Sampling Inc., USA) was used. The results are presented as gas volume per kilogram volatile solids at standard conditions (0°C, atmospheric pressure).

RESULTS AND DISCUSSION

The straw fraction of horse and cattle manure was separated and pretreated with 85% NMMO for 5 and 15h at 120ºC in order to open up the lignin shield and make the cellulose accessible for enzymatic degradation prior to biogas production. After the pretreatment, the NMMO was washed out, and the pretreated straw samples were dried in a freeze dryer and mixed with the rest of the manure samples and used for biogas production. The effect of the pretreatment was evaluated using anaerobic batch digestion assays. Moreover, the changes in the structure of the separated straw fraction due to the pretreatment were investigated by FTIR analysis.

Biogas Production

The biogas potential of horse and cattle manures mixed with the fraction of straw, before and after NMMO-pretreatment, was investigated in batch digestion experiments. Figure 2 shows the average values of accumulated methane production of triplicate samples measured during 52 days of incubation. The pretreatment improved the methane potential of every pretreated material. The methane yield increased by 22% and 53%, after the pretreatment of the straw fraction for 5 h and 15 h, respectively. The specific methane production for untreated cattle manure was 0.250 Nm^3 CH_4/kgVS, which increased to 0.305 Nm^3 CH_4/kgVS after 5 h pretreatment and further to 0.382 Nm^3 CH_4/kgVS after the 15 h pretreatment (Table 2). The same pattern was observed for the horse manure. The specific methane production increased to 0.350 and 0.422 Nm^3 CH_4/kgVS after 5 h, respective, 15 h pretreatments, while the methane yield of the untreated horse manure was 0.279 Nm^3 CH_4/kgVS. This means an increase in the methane yields by 25% and 51% for 5 h and 15 h pretreatments, respectively (Table 2).

The theoretical methane yield for manure samples was calculated using the general formula presented previously based on the fat, protein, and carbohydrate contents of the substrate (Davidsson 2007). According to the data presented at Table 1, the theoretical yield for cattle and horse manure was calculated to be 0.447 m^3 CH_4/kgVS and 0.445 m^3 CH_4/kgVS, respectively. These results are in accordance with the theoretical methane yield for dairy cattle manure of 0.469 m^3 CH_4/kgVS reported previously (Møller et al. 2004). These authors also calculated the theoretical yield of methane of manure mixed with straw, based on the composition of this mixture regarding to carbohydrates, lipids, and proteins and concluded that in comparison with manure without straw, 1 kg straw mixed with 100 kg manure would increase the yield of methane by approximately 10% considering the compositional variation in such biomass.

Table 2. Lignin and Carbohydrate Content, and Lateral Order Index (LOI) in IR Spectra of Straw Separated from Horse and Cattle Manure before and after Pretreatments with NMMO at 120ºC for 5 h and 15 h, Respectively. Specific methane yields and specific rate constants (k_0) were obtained during batch digestion of manure mixed with untreated *vs.* pretreated straw.

Sample	Total Lignin (% of TS)	Total Carbohydrates (%)	LOI[a]	Specific Methane Yield(Nm^3/kgVS)[b]	Specific rate constant k_0(day^{-1})
Horse manure					
untreated	32.92	44.68	2.68	0.279±0.002	0.071
5h-treatment	25.90	50.92	0.88	0.350±0.01	0.064
15h-treatment	22.72	53.84	0.66	0.422±0.05	0.086
Cattle manure					
untreated	39.53	25.44	5.36	0.250±0.09	0.041
5h-treatment	31.67	36.68	1.47	0.341±0.01	0.063
15h-treatment	29.75	38.68	1.26	0.382±0.08	0.072

[a] Lateral order index A1420cm^{-1}/A898cm^{-1}

[b] Accumulated methane per gram volatile solids produced after 52 days of incubation together with two standard deviations on accumulated methane production

[d] Specific rate constant during the first 12 days of incubation

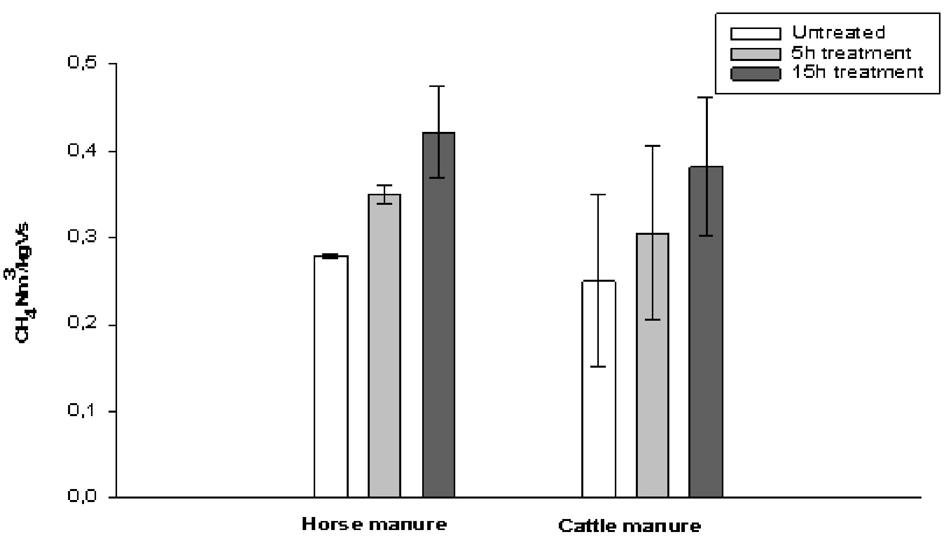

Fig. 2. Methane yield obtained after 52 days of anaerobic batch digestion from untreated and pretreated horse manure and cattle manure

A batch assay provides information about the methane yield from certain substrates as well as the kinetics of the degradation process. The results showed that not only the accumulated methane production, but also the degradation rate was improved as a result of the treatments. Figures 3a and 3b illustrate the variation of specific rate constant (k_0) for treated *vs.* untreated horse and cattle manures, respectively.

As shown in Fig. 3, k_0 increased with the pretreatment time for both the cattle and the horse manure samples (Table 2). After 15 h pretreatment, k_0 increased from 0.071 (untreated) to 0.086 d^{-1} in the case of horse manure, while similar treatment conditions resulted in an increase from 0.041 (untreated) to 0.072 d^{-1} for cattle manure. The pH was around 7 at the end of each digestion setup.

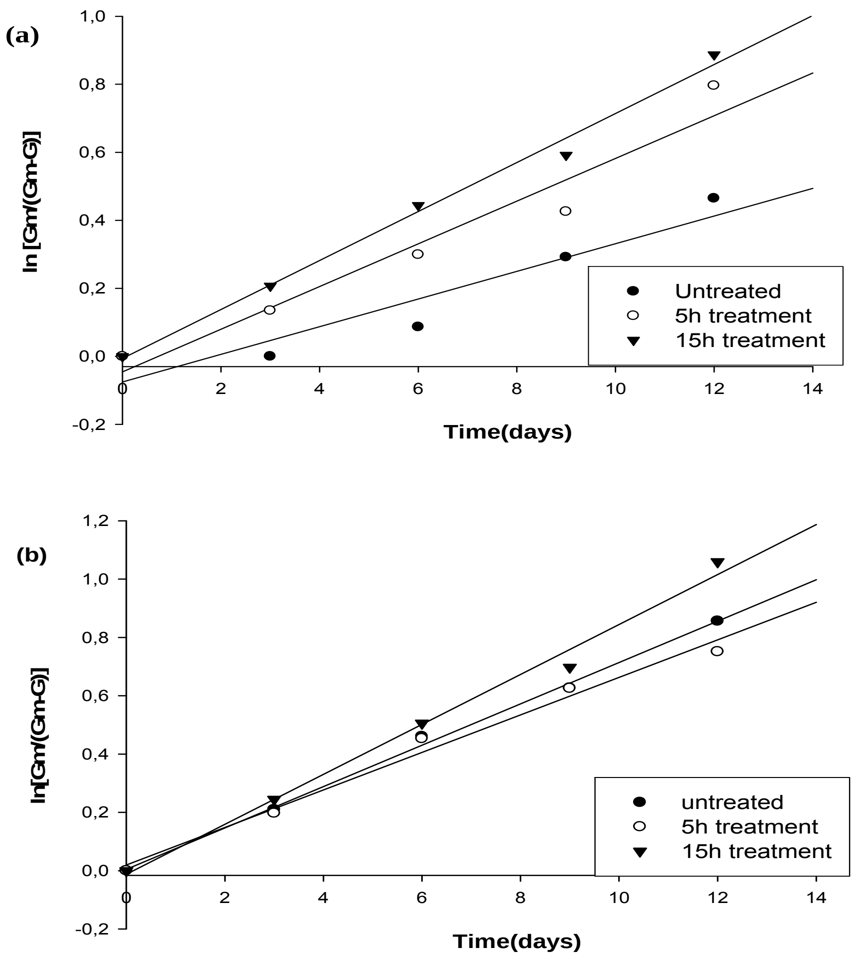

Fig. 3. Values of ln [*Gm/* (*G*m − *G)*] in function of time for (a) Untreated and pretreated cattle manure. (b) Untreated and pretreated horse manure

The Effects of Pretreatment on the Composition and Structure of Straw Separated from Manure

Lignin and carbohydrate contents of the untreated *vs.* pretreated straw fractions are shown in Table 2. The total lignin content (acid-soluble and insoluble) for untreated straw separated from cattle manure was 39.53%(w/w), which decreased to 31.67% and 29.75% following 5 h and 15 h pretreatment with NMMO, respectively. Consequently, the total carbohydrates of the straw from untreated cattle manure increased from 25.44% to 36.68% and 38.68% after the 5 and 15 h pretreatments, respectively. Similarly, with investigations of the straw separated from horse manure, a decrease in the lignin content was observed from 32.92 (wt %) to 25.90 (wt %) and to 22.72 (wt %) after 5 and 15 h pretreatments, respectively (Table 2). The total carbohydrate for untreated straw from horse manure was 44.68%, which increased to 50.92% and 53.84% after the pretreatments. This shows that the pretreatment reduced the structural lignin content by approximately 10% for both the separated straw samples and increased the carbohydrate content by 13% for straw separated from cattle manure and by 9% for that from horse manure. Additionally, an increase in the pretreatment time made the delignification more effective and further improved the following digestion process. This is because the pretreatment opened up the lignin that shields the cellulose and hemicelluloses, which in turn limits the accessibility of enzymes involved in further degradation during the following digestion process.

Table 3. Assignments of FT-IR Absorption Bands (cm^{-1}) with Related References

Bands (lit.) cm^{-1}	Assignment	Reference
3500-3100	OH –stretching vibrations	(Denise S. Ruzene 2007)
2919-2925	Methyl, methylene, and methine group vibrations	(Lawther et al. 1996)
2850,2920	CH2-streching bands	(Kristensen et al. 2008)
1727	Aliphatic carboxyl groups	(Buta, Zadrazil et al 1989)
1665-1680	Carbonylgroup (C=O) conjugated to aromatic ring	(MacKay, O'Malley et al. 1997)
1595-1605	Aromatic skeletal vibrations	(Buta, Zadrazil et al. 1989)
1595	Aromatic ring with C=O stretching	(MacKay, et al. 1997)
1505-1515	Aromatic skeletal vibrations	(Buta, Zadrazil et al. 1989)
1510	Aromatic ring with C—O stretching	(MacKay et al. 1997)
1420	Aromatic skeletal vibrations	(Buta, Zadrazil et al. 1989)
1245-1519	Guaiacyl and syringyl	(Niu, Chen et al. 2009)
1040	Dialkylether linkages linking cinnamyl alcohol subunits	(MacKay, et al. 1997)
1035	polysaccharide vibrations	(Lawther, Sun et al. 1996)

The change in the structure of the straws, caused by the pretreatment, was investigated by FTIR analysis. The interesting bands studied are summarized in Table 3, and the absorbance spectra are shown in Fig. 4. The comparison of these FTIR spectra shows that NMMO pretreatment resulted in reducing the absorption band around 1420 cm^{-1} and in increasing the absorption band at 898 cm^{-1}. These two bands are characteristic for the crystalline cellulose I and amorphous cellulose II, respectively (Nelson and O'Connor 1964). The crystallinity index, which is also called the lateral order index (LOI), was calculated as the absorbance ratio of the bands around 1420 and 898 cm^{-1} (He et al. 2008; Zhao et al. 2009). The results in Table 2 show that the crystallinity index decreases as the time of the pretreatment increases. This implies that there is a breakdown in the structure of straw, and as a result more sugars are hydrolyzed after the pretreatment, which improved the biogas production.

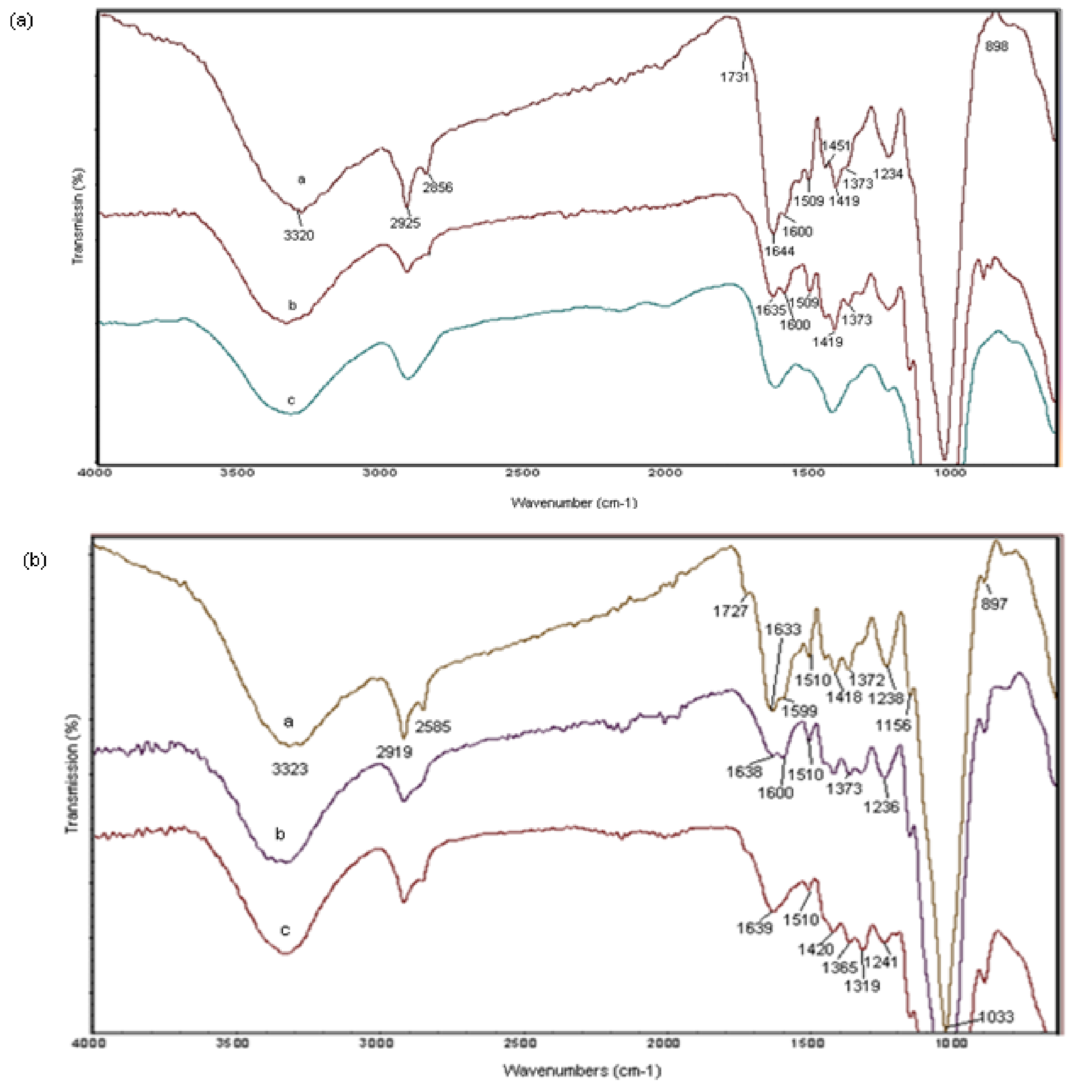

Fig. 4. FTIR spectrum of treated and untreated straw in (a) cattle manure and (b) horse manure (a) untreated, (b) 5 h treatment, (c) 15 h treatment

The lignin IR spectra have a strong broad band between 3500 and 3100 cm^{-1}, which is related to OH stretching vibrations caused by the presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds (Adney et al. 2008). The OH stretching band of the hydroxyl groups around 3300 cm^{-1} was changed to a higher wavenumber and somewhat broadened as a result of the pretreatment, which is an indication of weaker intra- and intermolecular hydrogen bonding and thereby a lower crystallinity (Jeihanipour et al. 2009). This result confirms the analysis data showing that the pretreatment reduced the structural lignin content in the straw (Table 2).

An additional effect of the pretreatment was the elimination of waxes, which can be observed from the reduced CH_2- stretching bands at about 2850 and 2920 cm^{-1} for the pretreated straw, suggesting a decrease in the amount of the aliphatic fractions of waxes (Kristensen et al. 2008). Several other changes were also observed in the structure, as is shown by changes in many other regions given in Table 3. These changes are more obvious as the pretreatment time increases (Fig. 4).

CONCLUSIONS

1. The aim of this study was the pretreatment of straw fraction separated from cow and horse manure, since the accumulation of this low digested lignocellulosic material can cause problems when manure is utilized for biogas production, and resulting in low methane yields.
2. NMMO pretreatment of straw separated from cattle and horse manure improved the methane yield during the following digestion of both manure substrates and these improvements were increased by increased the pretreatment times. Treating the straw fraction for 15 h increased the methane yield by 53% and 51% for cattle and horse manure, respectively, compared to that of when untreated straw was present in the manure samples.
3. The kinetics of the degradation process were evaluated using a specific rate constant, k_0, which was also improved when the straw fractions separated from both manure samples were pretreated for 15 h.
4. The effects of the pretreatment were evaluated by chemical and structural characterizations of the separated straw fractions. The total lignin content decreased by about 10% and the carbohydrate content increased by about 9% for straw separated from horse manure and by 13% for straw separated from cattle manure.
5. A reduction of crystallinity, obtained by FTIR, in the structure of the treated straw fractions, indicates an increase of the accessible surface area on the lignocellulosic material for further microbial degradation, improving the methane yield.

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