

AMMONOX: Ammonia for Enhancing Biogas Yield and Reducing NO_x—Analysis of Effects of Aqueous Ammonia Soaking on Manure Fibers

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Abstract Laboratory experiments have shown that aqueous ammonia soaking (AAS) is a promising treatment for increasing the methane yield of the solid fraction of manure (fibers). AMMONOX is a new concept based on the sustainable use of ammonia for enhancing biogas production at biogas plants digesting manure. The proposed process is based on an optimized AAS treatment of manure fibers in combination with an efficient ammonia recovery step. The enhancement of biogas production is achieved by enriching manure with AAS-treated fibers, or other lignocellulosic residues, while the ammonia recovered can be used for fulfilling the needs of the treatment itself. Excess of ammonia could be produced when ammonia is recovered from both the treated fibers and the digester effluent, which could be used for the reduction of NO_x in biogas-based electricity generation by gas turbines. In this survey study, the importance of different factors affecting the performance of AAS of digested manure fibers was investigated in order to conclude on which variables to optimize. Principal component analysis of the present data was used for a preliminary analysis of effects. The temperature and the ammonia concentration during AAS were the most influencing variables in terms of methane yield under the conditions tested. Further experiments should be conducted in order to investigate the effect of shorter AAS

duration than the ones tested (lower than 24 h) and for assessing the importance of the solid-to-liquid ratio in the treatment mixture; the follow-up campaign should be optimized with respect to possible interactions/correlated experimental factor effects.

Keywords Biogas · Pretreatment · Aqueous ammonia soaking · Methane yield · Manure fibers · Principal component analysis

Introduction

Livestock manure has been pointed out as one of the most important agricultural sources of environmental pollution. Manure is rich in valuable nutrients for plant growth such as nitrogen and phosphorus, which makes its use as a crop fertilizer and soil amendment a common practice in many countries. However, a large part of these nutrients are susceptible to loss to the environment through leaching, run-off or volatilization [1]. Due to the large contribution of manure to ammonia, greenhouse gas emissions and water pollution, concerns about its management have increased, and many regions in Europe that have an intensive livestock production, are struggling to find solutions in order to comply with the disposal limits stipulated in environmental legislation (91/676/EEC) [2].

Anaerobic digestion is one of the most attractive solutions for manure management, as it provides stabilization of nutrients as well as reduction of gas and odor emission. Moreover, the naturally produced methane is captured and can be used in the form of biogas as an alternative energy source. Financially the digestion of solely manure is however, a non-feasible process due to its low content in easily digestible organic matter [3]. This is because the easily digestible part of the

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animal feed has already been utilized in the animal digestion; thus manure contains a recalcitrant lignocellulosic concentrated part [4] mixed with washing water and other lignocellulosic biomasses, such as straw, that are used for bedding materials [5]. Consequently, manure-based biogas plants are forced to search for easily digestible organic materials, such as food waste, to be used for co-digesting with manure to ensure a cost-efficient process. Unfortunately, as the demand for these extra materials increases, their availability remains very limited, presenting biogas plants with a new problem [3].

As an alternative to co-digesting, pretreating the solid fraction (fibers) of manure could present a solution to its recalcitrant nature and enhance the methane yield when it is digested. Different manure separation techniques have been proposed [6], and several biogas plants are already equipped with a decanter centrifuge for this purpose [7]. The separation of the two fractions (liquid and solid) makes it possible to increase the final dry matter content of the material to digest, leading this way to an even higher biogas production per mass unit [8]. Various researchers have used different approaches for pretreating manure fibers in order to make them more easily degradable and increase the methane yield. According to a recent survey [9], aqueous ammonia soaking (AAS) has achieved the highest increase of methane yield of manure fibers, both of raw (separated from manure) and digested (separated from the effluent after a first digestion).

Ammonia has been used in the past for increasing the digestibility of straw for ruminants feeding [10], and only recently it has been used for improving the biofuels production. AAS is a very simple pretreatment that has so far been tested on some lignocellulosic biomasses for increasing ethanol production, and has recently captured the interest of researchers for biogas production. Apart from manure fibers, other biomasses that have been tested for this purpose include switchgrass [11], wheat straw [12–14], corn straw [15], rice straw [16] *miscanthus* and willow [13]. During the AAS pretreatment, the biomass in question is mixed with a water solution of ammonia and left over a certain period of time at mild temperatures (less than 90 °C). Subsequently, the ammonia is removed and the biomass can be used for digestion.

Despite the efficiency of AAS on increasing the methane yield of manure fibers, a significant variation of the performance of AAS under the conditions tested up to now has been observed. Jurado et al. [17] have performed AAS of digested manure fibers under different levels of temperature and duration achieving an increase of methane yield between 30 and 80 %. Further experiments with lower ammonia concentrations led to a higher increase of methane yield (up to 205 %) [9]. Batch experiments for the determination of the methane potential of AAS-pretreated raw manure fibers showed that AAS pretreatment increased the methane yield by 178 % compared to non-pretreated

fibers [17]. These results clearly show that AAS has a great potential for increasing the methane yield of manure fibers. Still, optimization of the most important parameters of AAS affecting the methane yield is necessary.

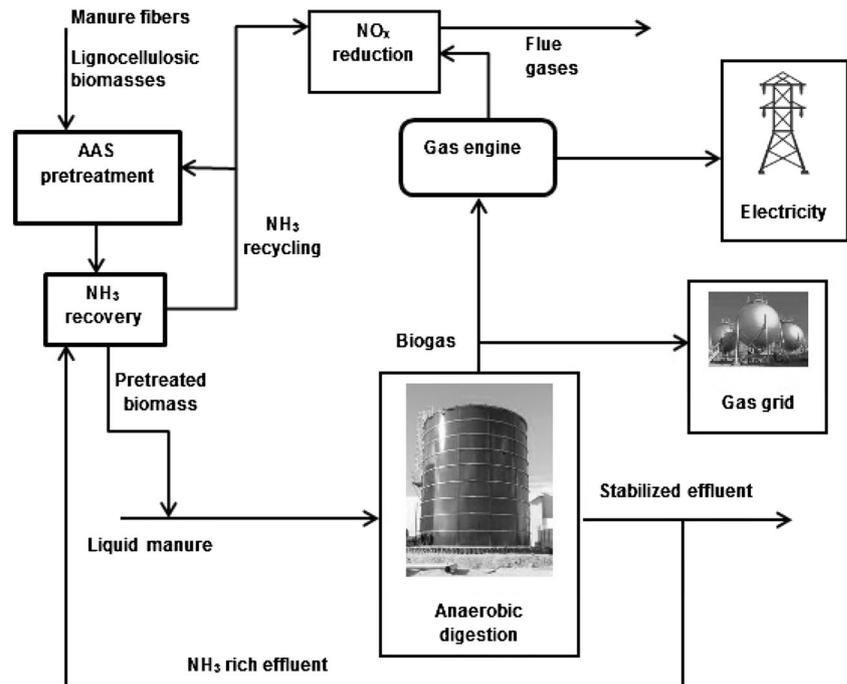
AMMONOX is a research project that aims to increase the efficiency of manure-based biogas production under an integrated process. The idea consists of an optimized ammonia treatment of manure fibers (or of other lignocellulosic biomasses) in combination with a successful ammonia recovery step (Fig. 1). As illustrated in Fig. 1, the manure fibers are first treated with a solution of aqueous ammonia under the conditions found to be optimal. Afterwards, the pretreatment mixture passes through a second process (Fig. 1) where the ammonia is removed until reaching a concentration low enough to avoid inhibition of the biological processes. After this step, the pretreated fibers are inserted to the digestion tank together with raw manure. The removal of ammonia is a relatively easy process due to its high volatility, thus providing an extra advantage of this pretreatment. This allows recovering ammonia and recycling it for fulfilling the chemical requirements of AAS, resulting in no extra consumption of chemicals. Additionally, as shown in Fig. 1, an excess of ammonia could be produced when the ammonia removal step includes both AAS-treated fibers and effluent from the anaerobic digestion step. This excess of ammonia can be used for the catalytic reduction of the NO_x produced, when the biogas is used for electricity generation by gas engines [18]. This is a commercially available technology and the ammonia required can be either in a gaseous form or in an aqueous solution [19].

High concentrations of ammonia are known to be inhibitory for the anaerobic digestion process, affecting mostly the methanogenesis step [20]. Thus after the AAS pretreatment, an ammonia removal step is essential for ensuring a stable process. Furthermore, the recovery of ammonia is of high importance for reducing the cost of the process as the ammonia can be recycled. Swine manure is often rich in ammonia, and during the anaerobic digestion process, degradation of proteins also takes place resulting to an increased ammonia concentration in the effluent. Some biogas plants are already equipped with ammonia-stripping technologies either for treating raw manure or for the post-treatment of the digestate prior to final disposal [7]. Nevertheless, the adequacy of ammonia-stripping of streams with such high ammonia concentrations as used in the AAS treatment has not been investigated yet. Moreover, the recovery of ammonia by stripping technology is achieved by means of acids, resulting to ammonium salts as final products. Therefore, modifications of stripping technology as well as additional methods should be considered for an efficient recovery of pure ammonia.

In summary, the three objectives of AMMONOX are:

- The optimization of the most important parameters of AAS affecting the methane yield of manure fibers, and

Fig. 1 General scheme of the AMMONOX concept for an enhanced manure-based biogas production in a sustainable way



of other lignocellulosic biomasses, for an enhanced manure-based biogas production,

- The identification and application of a successful ammonia removal technology that will permit to recycle the ammonia needed for AAS, and
- The use of the excess of ammonia recovered for the catalytic reduction of NO_x .

The focus of this study lays on the first objective of the AMMONOX process, which is considered to be crucial for identifying the adequate ammonia recovery technology and assessing the surplus of ammonia produced for the NO_x reduction. In order to develop a process for optimizing the AAS treatment in terms of maximum methane yield, some preliminary analyses were conducted and the results are presented in this study. A data exploration method was used for uncovering the possibly hidden information from the experimental data obtained so far, during studies of the effect that different conditions of AAS treatment of digested manure fibers had on methane yield.

Materials and Methods

Data Set

All the experimental data used in this analysis originate from research at Aalborg University and are published in Jurado et al. [17] and Mirtsou-Xanthopoulou et al. [9]. The data consist of CH_4 yields of digested swine manure fibers

pretreated with AAS under two different temperatures (22 and 55 °C), three different pretreatment durations (1, 3 and 5 days) and six different NH_3 concentrations of the reagent (5, 10, 15, 20, 25 and 32 % w/w). All pretreatment mixtures were further used for experiments after a distillation step where the NH_3 concentration was reduced to below-inhibition levels [9]. Two data sets were formed for the data exploration analysis, both originating from the same experiments. The first data set, presented in Table 1, concerned ultimate CH_4 yields (CH_4 yield when no further gas production was detected, usually more than 30 days) of biochemical methane potential (BMP) tests; and the second data set concerned the CH_4 yields after approximately 18 days from the same experiments. The purpose of analyzing the two different data sets was to assess the effect of the AAS parameters on the short term CH_4 yield and on the ultimate CH_4 yield. Both data matrixes were constructed by 54 rows, corresponding to the total amount of experiments (including triplicates), and 4 columns, corresponding to the pretreatment variables (temperature, duration and NH_3 concentration of the reagent) and to the CH_4 yield obtained under these conditions.

Principal Component Analysis

Principal component analysis (PCA) was used for data exploration purposes and for identifying tendencies of the CH_4 yield based on the different levels of the pretreatment variables. The software used for this purpose was The

Table 1 Data matrix of ultimate CH₄ yields of digested manure fibers treated with AAS under different conditions used for PCA^a

No. of experiment	Temperature of AAS (°C)	Duration of AAS (days)	NH ₃ concentration of AAS (% w/w)	CH ₄ yield (ml CH ₄ /g TS)
1a	22	1	32	106.2243
1b	22	1	32	111.5652
1c	22	1	32	107.0878
2a	22	3	32	143.8944
2b	22	3	32	128.7069
2c	22	3	32	141.5351
3a	22	5	32	139.7697
3b	22	5	32	126.7412
3c	22	5	32	128.7438
4a	55	1	32	110.8356
4b	55	1	32	119.3204
4c	55	1	32	110.6114
5a	55	3	32	125.8958
5b	55	3	32	134.3595
5c	55	3	32	132.4131
6a	55	5	32	119.7610
6b	55	5	32	124.3537
6c	55	5	32	124.6628
7a	22	3	32	144.0650
7b	22	3	32	127.2090
7c	22	3	32	163.6780
8a	22	3	25	163.3950
8b	22	3	25	153.7280
8c	22	3	25	187.3500
9a	22	3	20	181.0820
9b	22	3	20	180.0060
9c	22	3	20	144.2920
10a	22	3	15	145.1680
10b	22	3	15	177.6820
10c	22	3	15	167.3930
11a	22	3	10	146.3340
11b	22	3	10	171.3740
11c	22	3	10	177.0590
12a	22	3	5	166.8230
12b	22	3	5	165.6650
12c	22	3	5	156.4660
13a	22	1	25	218.9580
13b	22	1	25	202.4960
13c	22	1	25	217.1620
14a	22	3	25	205.0210
14b	22	3	25	227.4380
14c	22	3	25	222.6920
15a	22	5	25	158.9800
15b	22	5	25	178.3310
15c	22	5	25	162.6690
16a	22	1	5	169.4050
16b	22	1	5	201.0910
16c	22	1	5	240.9190

Table 1 continued

No. of experiment	Temperature of AAS (°C)	Duration of AAS (days)	NH ₃ concentration of AAS (% w/w)	CH ₄ yield (ml CH ₄ /g TS)
17a	22	3	5	197.9670
17b	22	3	5	191.9950
17c	22	3	5	209.1050
18a	22	5	5	205.2330
18b	22	5	5	185.5600
18c	22	5	5	196.9940

^a Data graphically presented in Jurado et al. [17] and Mirtsou-Xanthopoulou et al. [9]

Unscrambler[®]X 10.3 (CAMO, Norway). Standardization of the data matrixes was performed by first subtracting the mean from the values and then dividing them by the standard deviation of the corresponding variable. This way, the variables that initially have a very different range of variance become more comparable as the variance becomes even.

Results and Discussion

During the last years, different pretreatments have been tested on manure fibers (both raw and digested), for increasing the biogas or methane yield of manure-based anaerobic digestion systems. Hartmann et al. [21] tested the mechanical maceration of manure and found a 25 % increase on biogas production while the resulted increase was insignificant when only raw manure fibers were treated. Raphique et al. [22] tested a thermal, a thermochemical and a chemical [with Ca(OH)₂] pretreatment of raw manure fibers and reported a 28 %, a 72 % and a negative increase of CH₄ yield respectively. In another study, thermal steam explosion was tested on raw manure fibers and resulted in a 50 % increase of CH₄ yield [23]. While the highest increase of CH₄ yield achieved up to now from pretreated raw manure fibers is 178 % by AAS [17], pretreating digested manure fibers has proved to be more efficient. Angelidaki and Ahring [24] reported a 17 % increase of biogas potential from digested manure fibers treated by mechanical maceration, and a 30 % increase when treated with a biological treatment. Bruni et al. [25, 26] investigated the effects of a mechanical, a chemical, a thermal and an enzymatic pretreatment of digested manure fibers and found that the most significant CH₄ increase was generated by steam treatment with H₂SO₄ (67 % increase) and treatment with CaO (66 % increase). Finally, wet explosion of digested manure fibers resulted in a 136 % increase of CH₄ yield [27]. In comparison to the results obtained so far from these pretreatments, AAS of digested manure fibers has achieved an increase of CH₄ yield up to 205 % (265 % at 17 days of digestion) when compared to non-

treated fibers [9]. In order to identify the important factors that affected the performance of AAS on digested manure fibers, a statistical tool was used for analyzing the results obtained up to now.

PCA is an exploratory data analysis method aiming at separating the important information hidden in one data matrix from the noise [28]. In PCA, a new orthogonal axis-system is defined in a way that each principal component (PC) represents an axis, along which, the maximum variation within the data is described. The first PC (PC1) is defined by the maximum variance direction (axis) of the data set; the second PC (PC2) is orthogonal to PC1 modeling the second maximum variance direction, and so on. The transformed data, scores, can be plotted in the new PC space (score plot). Interpretation of the variance modeled by each PC allows statements as to the reasons why the data are distributed as revealed in the score space, and is assessed by the loadings relationships (loadings plot), in which the contribution of the initial variables on the construction of PCs can be assessed. This way the hidden data structure, e.g. groupings, clusters, trends between objects (score relationships) and the correlation of variables responsible (loading relationships) is revealed.

PCA was conducted for exploring the effects of temperature, duration and NH₃ concentration of the AAS pretreatment of digested manure fibers on CH₄ yield as resulted from batch tests. The first data set concerns ultimate CH₄ yields obtained from BMP tests under different conditions of AAS as described earlier. In Fig. 2 the scores plot of the first data set is shown. The correspondence of the names of the samples to the conditions of the AAS pretreatment is given in Table 1. In Fig. 3 the loadings plot is presented where the correlation of the different variables can be viewed and from where assistance can be provided for the interpretation of the scores plot.

As revealed from the loadings plot (Fig. 3), the first PC explains 50 % of the total data variance and models three of the variables (CH₄ yield, temperature, and NH₃ concentration) while the second PC models an additional 25 % of the variance, expressing only one variable, the duration of the AAS. As observed in the loadings plot (Fig. 3), CH₄

Fig. 2 Scores plot of the PCA of experiments with AAS pretreated digested manure fibers under different temperature, duration and NH_3 concentration of the reagent. The plot shows the correlation of the experiments in the PC1 versus PC2 space. The numbers of the experiments are explained in Table 1

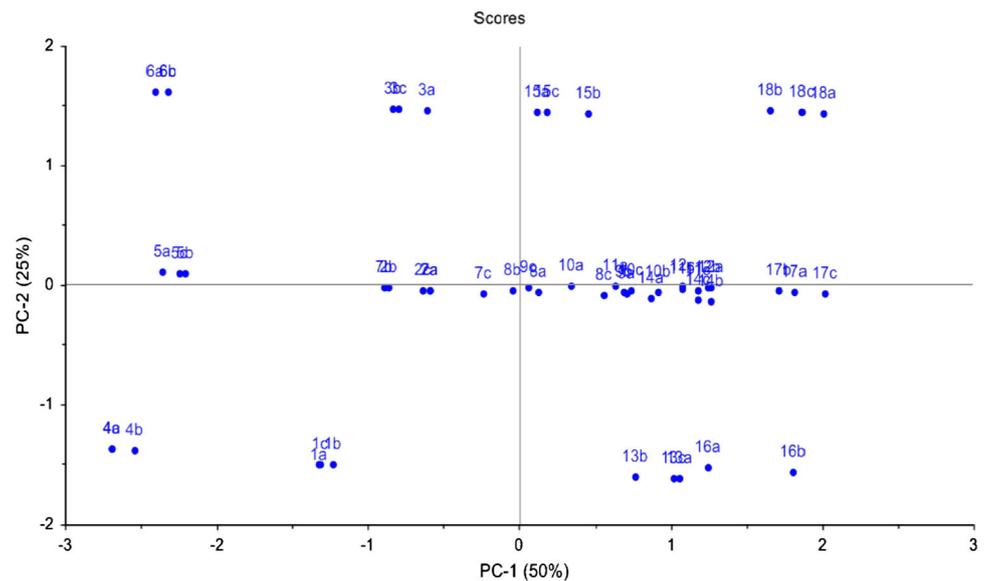
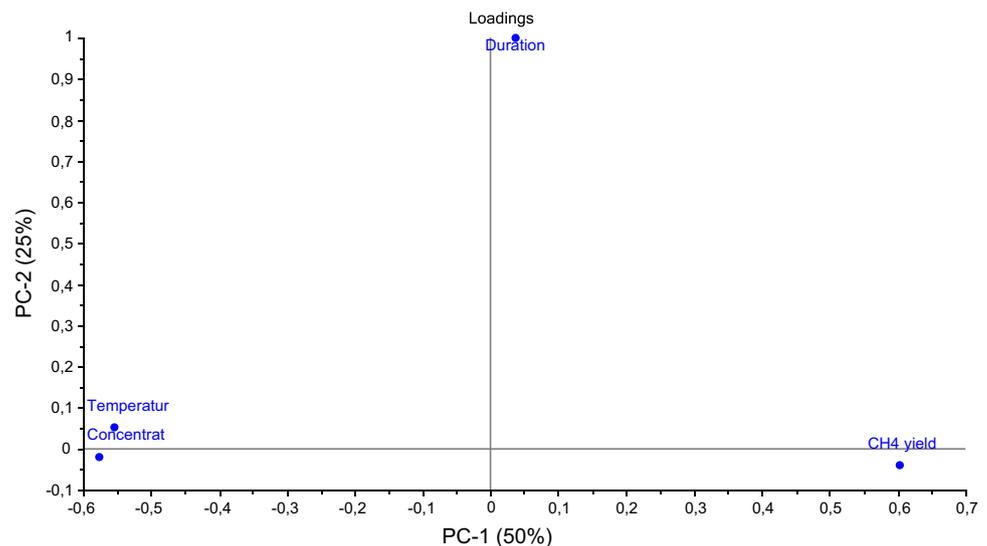


Fig. 3 Loadings plot of the PCA of experiments with AAS treated digested manure fibers. The loadings plot shows the correlation of the four variables (temperature, NH_3 concentration, duration and CH_4 yield) of the experiments in the PC1 versus PC2 space



yield seems to be strongly, negatively correlated to both temperature and NH_3 concentration. On the other hand, the duration of the pretreatment seems not to be correlated to the CH_4 yield, as duration varies along PC2 and not along PC1. This can also be concluded from the scores plot in Fig. 2. PC2 clearly separates the batches according to duration; along this PC (from negative to positive values) the duration increases from 1 to 5 days, forming three horizontal groupings. On the other hand PC1 separates samples according to temperature of the pretreatment, high temperature ($55\text{ }^\circ\text{C}$) samples are situated on the extreme left, and according to NH_3 concentration, samples from the left to the right generally present a decreasing NH_3 concentration.

Based on the position of CH_4 yield on the loadings plot illustrated in Fig. 3, the highest CH_4 yields correspond to

the samples on the right of the scores plot. These samples were all treated at room temperature ($22\text{ }^\circ\text{C}$) and at all different durations (1, 3 and 5 days). Regarding the NH_3 concentration with which these samples were treated, although the general trend is that the concentration decreases along PC1, the different levels within the group of samples on the right of the plot are difficult to distinguish (Fig. 2). This might be due to the fact that not all different concentration levels were tested on all different temperature and duration combinations due to logistical constraints; this data set may therefore not have been optimal for describing all main as well as interacting effects between concentration and the rest of variables. On the contrary it is safe to conclude that the highest level of concentration has the lowest effect on increasing CH_4 yield

as all samples treated with 32 % w/w NH_3 (aq.) are situated on the left of the scores plot. PCA of the second data set (CH_4 yield at ca. 18 days) has shown the same correlations (results not shown), which means that the influence of these factors on CH_4 yield at the ranges tested, does not change over the duration of digestion.

Few studies have been conducted assessing the performance of AAS on lignocellulosic biomasses under different conditions. Regarding the importance of NH_3 concentration used for the pretreatment, literature seems to be ambiguous. Li et al. [14] have tested AAS on wheat straw under 0–30.8 % w/v NH_3 (aq.) and found that the CH_4 yield increased when the NH_3 concentration increased up to 18 % but not when it was further increased. Song et al. [15, 16] reported that increasing the ammonia concentration from 1 to 4 % w/w and up to 10 % w/w led to an enhanced CH_4 yield from pretreated rice straw and corn straw respectively. On the other hand, Ko et al. [29] mentioned no significant effect on enzymatic digestibility of pretreated rice straw when increasing the NH_3 concentration from 12 to 28 % w/w, and actually reported a decrease of digestibility when the highest NH_3 concentration was applied. Other studies have shown a very slight increase of digestibility of the treated biomass or of CH_4 yield when the concentration was increased [12, 30, 31]. The importance of the NH_3 concentration of the pretreatment might be attributed to the different pH along the different concentrations of NH_3 (aq.) solutions, as these two factors are strongly correlated. According to this and to the conclusions derived from this PCA, the concentration of NH_3 used for the pretreatment seems to be an important though not a decisive factor on the success of AAS, except when concentration of NH_3 is very high resulting to a negative effect.

In agreement to this survey, Li et al. [14] found the temperature of the pretreatment to be very influencing on the CH_4 yield. They mention a positive correlation between these two variables, attributing this observation to a higher degree of delignification. In general, an increase of temperature has been linked to a higher lignin removal, which is often associated to a higher digestibility of lignocellulosic materials. A strong correlation between digestibility and lignin removal has been observed in more studies [29, 32]. Nonetheless, studies on compositional changes of manure fibers revealed that no apparent lignin removal had taken place after the AAS pretreatment [33]. The authors suggest that the increased CH_4 yield is probably a result of the increased exposure of cellulose, resulted from the swelling that AAS caused. This theory is in accordance with previous works stating that ammonia treatment causes a “fiber expansion” that facilitates the enzymatic hydrolysis of lignocellulosic biomasses [34]. Moreover, it has been reported that enhanced methane yield could result

from very small changes on lignin matter rather than only when delignification takes place [14].

The different observations on the effect of ammonia on lignocellulosic structures might be attributed to the different solid-to-liquid (S:L) ratios used for the pretreatment. In the case of pretreated manure fibers the S:L ratio was ca. 1:3, [1 g material: 2.8 ml NH_3 (aq.)] if considering that the treatment was performed with 10 ml NH_3 (aq.) per 1 g TS and the TS content of digested fibers was ca. 28 % [9, 17]. This ratio is much higher than the ratios of 1:6 and 1:10 that are usually used for other biomasses [14, 32, 34]. The S:L ratio has been pointed out as a very influencing factor for NH_3 pretreatment of lignocellulosic biomasses [35] and previous studies have shown that a decrease of the S:L ratio causes an increase of delignification and of enzymatic digestibility. Yoo et al. [31] studied the effect of three different S:L ratios of AAS on barley straw (i.e. 1:3, 1:6 and 1:10) and found that a decrease of the S:L ratio resulted to higher delignification and higher enzymatic digestibility. Similar results were obtained on a study of AAS on corn stover at S:L ratios from 1:2 to 1:10 [30]. In conclusion, AAS at high S:L ratios may affect lignocellulosic biomasses mainly by a different mechanism (swelling) than removing the lignin and this might be the reason why increasing temperature did not cause an increase of the CH_4 yield from pretreated manure fibers. For a deeper understanding of the nature of the AAS pretreatment, it is essential to test this hypothesis in further investigations.

Finally, some studies have reported the duration of AAS to be important in terms of enzymatic digestibility of lignocellulosic biomasses. Kim and Lee [30] and Yoo et al. [31] tested different durations of AAS on corn stover and barley straw respectively and both groups concluded that increasing the duration had a positive effect only up to 12 h, and then remained stable. Although in both cases the rest of the variables were fixed at constant values, strong interaction effects exist between duration and the two variables (which agrees with Chandel et al. [36]), with the interaction of duration and concentration to be the most important. In all these studies, the effect of pretreatment duration was examined only at high temperatures (over 50 °C) and high NH_3 concentrations (15–28 %) and in contrast to the data set examined at the present study, the ranges tested included also lower durations (down to 6 h). This indicates that reducing the pretreatment duration to a few hours might increase considerably the importance of this factor.

Interactions between the AAS parameters have been mentioned in more studies, and given the nature of the variables it is well expected that correlations exist. Low temperature is expected to increase the importance of duration and vice versa [34]. Nevertheless, AAS on different lignocellulosic biomasses seems to act with a different mechanism and conclusions on linear interactions between

the variables are probably precipitous. For instance, low temperature (up to 55 °C) of AAS on manure fibers did not result to a higher importance of the duration of the pretreatment as discussed earlier. This might be due to a parallel interaction with the NH_3 concentration of the pretreatment or more likely with the S:L ratio. Additionally, it has to be taken into account that the composition of manure fibers is considerably different from the composition of the different straws that have been tested up to know, mainly due to their much higher moisture content (usually around 70 %). Thus, it could be hypothesized that the duration of the pretreatment may become important if the test range widens up to higher values. However, very high durations might not be of interest for industrial applications as they are not economically favorable. The selection of the ranges within the variables that will be optimized should be chosen very carefully and after taking into consideration all possible limitations derived from a full scale application.

Given the uncertainty of the mechanism of AAS under the whole range of each variable, and the complexity of a multivariable system, a suitable experimental design is necessary in order to conclude on the possible interactions between the pretreatment variables affecting the CH_4 yield of pretreated manure fibers. There appear to be four most influential factors, i.e. temperature, NH_3 concentration, AAS duration and the S:L ratio. Instead of standard experimental design which requires a substantial total number of runs if all possible interactions shall be covered (three levels, four factors), an alternative design that will allow outlining the full impact of all potential interactions would be more appropriate, i.e. a so-called random design, as outlined in [28]. This design facilitates yield modeling using partial least squares regression based on a reduced number of runs, sacrificing formal ANOVA significance evaluation for direct interaction-and-main effects influences as revealed in pertinent loading-weights relationships. Based on this first foray, final optimization is based only on the most influential factors and the interactions revealed; an efficient two-stage optimization.

Conclusions

AAS has been recently proved to be a pretreatment of great potential for increasing CH_4 yield of manure fibers. The AAS pretreatment of manure fibers coupled with a successful NH_3 recovery step could lead to a more sustainable biogas production allowing biogas plants to operate solely on manure. Furthermore, integration of the digester effluent to the NH_3 recovery step could produce an excess of NH_3 to be used for the reduction of NO_x , providing this way a

more environmental friendly operation of gas engines when the biogas produced is used for electricity generation.

In this study, the performance of AAS as a pretreatment of digested manure fibers under different conditions was explored in terms of maximum CH_4 yield, based on previous experiments. PCA revealed that within the ranges of pretreatments and combinations tested, temperature and NH_3 concentration have more influence on CH_4 yield, while the duration of AAS is less important. Optimization of AAS of manure fibers must be conducted for guaranteeing maximum performance, and the effect of lower values of NH_3 concentration and duration should be included in the investigation. The most important factors of AAS that seem to affect the performance of the pretreatment and should be optimized are temperature, NH_3 concentration, duration and S:L ratio. It will be necessary to establish an experimental design that will allow a full impact also from interactions between these four factors, i.e. a so-called random design.

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References

- Oenema, O., Oudendag, D., Velthof, G.L.: Nutrient losses from manure management in the European Union. *Livest. Sci.* **112**, 261–272 (2007)
- Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources
- Møller, H.B., Nielsen, A.M., Nakakubo, A., Olsen, H.J.: Process performance of biogas digesters incorporating pre-separated manure. *Livest. Sci.* **112**, 217–223 (2007)
- Triolo, J.M., Ward, A.J., Pedersen, L., Sommer, S.G.: Characteristics of animal slurry as a key biomass for biogas production in Denmark. In: Matovic, M.D. (ed.) *Biomass Now—Sustainable Growth and Use*, pp. 307–326. InTech, Croatia (2013)
- Foged, H.L. (ed.): *Technologies for handling of livestock manure. Livestock Manure to Energy—Status, Technologies and Innovation in Denmark*, pp.16–17. Agro Business Park A/S, Tjele (2012)
- Burton, C.H.: The potential contributions of separation technologies to the management of livestock manure. *Livest. Sci.* **112**, 208–216 (2007)
- Frandsen, T.Q., Rodhe, L., Baky, A., Edström, M., Sipilä, I., Petersen, S.L., Tybirk, K. (ed.): *Best Available Technologies for Pig Manure Biogas Plants in the Baltic Sea Region*, pp. 159. Baltic Sea 2020, Stockholm (2011)
- Asam, Z., Poulsen, T.G., Nizami, A., Raphique, R., Kiely, G., Murphy, J.D.: How can we improve biomethane production per unit of feedstock in biogas plants? *Appl. Energy* **88**, 2013–2018 (2011)

9. Mirtsou-Xanthopoulou, C., Jurado, E., Skiadas, I.V., Gavala, H.N.: Effect of aqueous ammonia soaking on the methane yield and composition of digested manure fibers applying different ammonia concentrations and treatment durations. *Energies* **7**, 4157–4168 (2014)
10. Horton, G.M.J., Steacy, G.M.: Effect of anhydrous ammonia treatment on the intake and digestibility of cereal straws by steers. *J. Anim. Sci.* **48**, 1239–1249 (1979)
11. Himmelsbach, J.N., Raman, D.R., Anex, R.P., Burns, R.T., Faulhaber, C.R.: Effect of ammonia soaking pretreatment and enzyme addition on biochemical methane potential of switchgrass. *Trans. ASABE* **53**(6), 1921–1927 (2010)
12. Hashimoto, A.G.: Pretreatment of wheat straw for fermentation to methane. *Biotechnol. Bioeng.* **28**, 1857–1866 (1986)
13. Jurado, E., Gavala, H.N., Skiadas, I.V.: Enhancement of methane yield from wheat straw, *miscanthus* and willow using aqueous ammonia soaking. *Environ. Technol.* **34**(13–14), 2069–2075 (2013)
14. Li, Y., Merrettig-Bruns, U., Strauch, S., Kabasci, S., Chen, H.: Optimization of ammonia pretreatment of wheat straw for biogas production. *J. Chem. Technol. Biotechnol.* (2014). doi:10.1002/jctb.4297
15. Song, Z., Yang, G., Liu, X., Yan, Z., Yuan, Y., Liao, Y.: Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. *PLoS One* **9**(4), e93801 (2014). doi:10.1371/journal.pone.0093801
16. Song, Z., Yang, G., Guo, Y., Zhang, T.: Comparison of two chemical pretreatments of rice straw for biogas production by anaerobic digestion. *BioResources* **7**(3), 3223–3236 (2012)
17. Jurado, E., Skiadas, I.V., Gavala, H.N.: Enhanced methane productivity from manure fibers by aqueous ammonia soaking pretreatment. *Appl. Energy* **109**, 104–111 (2013)
18. Li, J.H., Chang, H.Z., Ma, L., Hao, J.M., Yang, R.T.: Low-temperature selective catalytic reduction of NO_x with NH₃ over metal oxide and zeolite catalysts—a review. *Catal. Today* **175**(1), 147–156 (2011)
19. Nakajima, F., Hamada, I.: The state-of-the-art technology of NO_x control. *Catal. Today* **29**, 109–115 (1996)
20. Chen, Y., Cheng, J.J., Creamer, K.S.: Inhibition of anaerobic digestion process: a review. *Bioresour. Technol.* **99**, 4044–4064 (2008)
21. Hartmann, H., Angelidaki, I., Ahring, B.K.: Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration. *Water Sci. Technol.* **41**(3), 145–153 (2000)
22. Raphique, R., Poulsen, T.G., Nizami, A.S., Asam, Z., Murphy, J.D., Kiely, G.: Effect of thermal, chemical and thermo-chemical pre-treatments to enhance methane production. *Energy* **35**, 4556–4561 (2010)
23. Ferreira, L.C., Souza, T.S.O., Fdz-Polanco, F., Pérez-Elvira, S.I.: Thermal steam explosion pretreatment to enhance anaerobic biodegradability of the solid fraction of pig manure. *Bioresour. Technol.* **152**, 393–398 (2014)
24. Angelidaki, I., Ahring, B.K.: Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Sci. Technol.* **41**(3), 189–194 (2000)
25. Bruni, E., Jensen, A.P., Angelidaki, I.: Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. *Bioresour. Technol.* **101**, 8713–8717 (2010)
26. Bruni, E., Jensen, A.P., Angelidaki, I.: Steam treatment of digested biofibers for increasing biogas production. *Bioresour. Technol.* **101**, 7668–7671 (2010)
27. Biswas, R., Ahring, B.K., Uellendahl, H.: Improving biogas yields using an innovative concept for conversion of the fiber fraction of manure. *Water Sci. Technol.* **66**, 1751–1758 (2012)
28. Esbensen, K. (ed.): Principal component analysis (PCA)—introduction. *Multivariate Data Analysis-in Practice* 5th edn, pp. 19–74. CAMO Software AS, Oslo (2001)
29. Ko, J.K., Bak, J.S., Jung, M.W., Lee, H.J., Choy, I., Kim, T.H.: Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresour. Technol.* **100**, 4374–4380 (2009)
30. Kim, T.H., Lee, Y.Y.: Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. *Appl. Biochem. Biotechnol.* **136–140**, 81–92 (2007)
31. Yoo, C.G., Nghiem, N.P., Hicks, K.B., Kim, T.H.: Maximum production of fermentable sugars from barley straw using optimized soaking in aqueous ammonia (SAA) pretreatment. *Appl. Biochem. Biotechnol.* **169**, 2430–2441 (2013)
32. Liu, Z., Padmanabhan, S., Cheng, K., Schwyter, P., Pauly, M., Bell, A.T., Prausnitz, J.M.: Aqueous-ammonia delignification of *miscanthus* followed by enzymatic hydrolysis to sugars. *Bioresour. Technol.* **135**, 23–29 (2013)
33. Jurado, E., Hansen, M.T.A., Gavala, H.N., Skiadas, I.V.: Compositional changes in swine manure fibers treated with aqueous ammonia soaking (AAS) resulting in increased methane potential. In: Proceedings of the 13th world congress on anaerobic digestion, Santiago de Compostela, Spain (2013)
34. Gao, A.H., Bule, M.V., Laskar, D.D., Chen, S.: Structural and thermal characterization of wheat straw pretreated with aqueous ammonia soaking. *J. Agric. Food Chem.* **60**, 8632–8639 (2012)
35. Hedayatkah, A., Motamedi, H., Najafzadeh, H., Varzi, N., Chezelbash, G., Bahnamiry, M.A., Karimi, K.: Improvement of hydrolysis and fermentation of sugarcane bagasse by soaking in aqueous ammonia and methanolic ammonia. *Biosci. Biotechnol. Biochem.* **77**(7), 1379–1383 (2013)
36. Chandel, A.K., Antunes, F.A.F., da Silva, M.B., Silva, S.S.: Unraveling the structure of sugarcane bagasse after soaking in concentrated aqueous ammonia (SCAA) and ethanol production by *Scheffersomyces (Pichia) stipitis*. *Biotechnol. Biofuels* **6**, 102 (2013)