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## APPLICATION OF AQUEOUS AMMONIA SOAKING FOR ENHANCEMENT OF METHANE POTENTIAL OF SWINE MANURE FIBERS

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### Abstract

**Purpose:** Increasing the methane productivity of manure based biogas plants is challenging because the solid fraction of manure contains lignocellulosic fibers, which are difficult to biodegrade and thus make anaerobic digestion process slow and economically unfavourable. Therefore, pretreatment of the solid fraction is a prerequisite for increasing its digestibility. The purpose of the present study was to evaluate aqueous ammonia soaking (AAS) and subsequent ammonia removal as a pretreatment method for increasing methane potential and biogas productivity of raw and digested manure fibers.

**Methods:** Manure fibers were pretreated with AAS for 3 days at 22°C and methane production was evaluated in batch experiments (methane potential tests).

**Results:** It was proven that AAS altered the lignocellulosic structure increasing significantly the concentration of soluble organic material. AAS pretreatment resulted at a 40-80% and 170% increase in methane yield from digested and raw manure fibers, respectively. Moreover, methane potential was evaluated at different organic loadings to test potential inhibition phenomena. Four different TS loadings were tested: 0.16, 0.25, 0.5 and 1 g TS per 10 ml of inoculum. No inhibition was observed for the digested fibers at the loadings tested while raw fibers exhibited slight inhibition only at very high loadings.

**Main conclusions:** In the present study, AAS was successfully applied as a pretreatment method to increase methane potential of swine manure fibers. Batch anaerobic digestion of AAS-treated digested manure fibers could stand loadings as high as 100 g TS / L inoculum with no inhibition problems.

## 1- INTRODUCTION

Biogas production and utilization has become a major part of the rapidly growing renewable energy sector. In recent years, biogas technology has progressed significantly and its application has experienced an explosive growth worldwide in municipalities, industry and agriculture. Currently, most agricultural biogas plants are used to ferment liquid manure [1]. The use of swine manure in biogas production is a common practice in Denmark. However, biogas plants digesting liquid manure alone are not economically viable due to the relatively low organic content of the manure, usually 3-5%. Therefore, current biogas production in Denmark is based on at least 75% animal manure and up to 25% additional biomasses characterized by high methane potential (e.g. slaughterhouse wastes, glycerine, crops, animal fat, fish oil, etc) in order to increase the methane efficiency and thus the process profitability [1]. However, due to the increased demand for biomass feedstock in the bioenergy sector, supply of alternative organic fractions at reasonable prices is becoming increasingly limited which directly affects the income and overall economy of the biogas plants. Moreover, the frequent change of additional biomass supply results in frequent adaptation of the anaerobic digestion processes and thus reduced the overall biogas efficiency. Two possible alternatives have been studied most recently in order to solve this problem: a) the addition of alternative materials to increase dry matter concentration, such as wheat straw [2], which creates a dependency on another extra material and b) the development and application of solid-liquid separation technologies [3]. The solid-liquid separation has the advantage of more efficient and cheaper transportation since only the solid fraction of the manure will be transported to the biogas plant while the liquid fraction will remain in the farm, where it will be used as fertilizer [4].

The solid fraction of manure consists mainly of swine faeces, lignocellulosic plant fibers and some additional elements (as hair, skin, and soil from roughage). Biogas production from manure fibers presents difficulties mainly due to the rigid lignocellulosic structure [5]. Therefore, pretreatment is a prerequisite process for accessing fermentable carbohydrates. The main purpose of pretreatment is disrupting the lignocellulosic matrix, facilitating the hydrolysis of cellulose and hemicellulose by cellulases and/or xylanases produced by cellulolytic and xylanolytic microorganisms and allowing thus the subsequent anaerobic fermentation and methanogenesis steps. Several pretreatment methods for increasing the biodegradability of methane production from fibers and other lignocellulosic materials have been reported. Mechanical treatment, such as milling, increases the surface available for enzymatic attack and has been proven effective in increasing methane yields of lignocellulosic substrates up to 25%. Chemical treatment with acids, bases and oxidants has also been tested. Among them, alkaline hydrolysis with NaOH has been proven a satisfactory treatment when applied on lignocellulosic materials like straw [6].

In the present study, aqueous ammonia soaking (AAS) and subsequent ammonia removal has been used as a pretreatment method to increase methane potential and biogas productivity of manure fibers. AAS has been so far tested for bioethanol and chemicals production with satisfactory results [7-11]. AAS presents certain advantages as a pretreatment method; Ammonia is relatively safe to handle, non-polluting and non-corrosive and can be easily recovered due to its high volatility [5]. In comparison with high temperature ammonia pretreatment, AAS is characterized by low energy input, no formation of toxic compounds and no loss of sugars. However, studies on the effect the AAS has on methane production from various biomasses are scarce with just those of Himmelsbach et al. [12] and Jurado et al. [13] found so far in the international literature. In the present study, AAS was applied to both raw and digested (before and after anaerobic digestion) swine manure fibers showing an impressive methane potential increase. It has to be emphasized that apart the increased methane yield, the ammonia used for the pretreatment can be easily recycled in a full-scale

plant resulting in actually no chemicals consumption. Specifically, application of AAS on manure fibers in biogas plants already equipped with ammonia removal infrastructure will constitute a cost-efficient and sustainable pretreatment (or post-treatment) option.

## 2- MATERIALS AND METHODS

*Substrates and reagents.* Manure fibers were collected and stored at -20°C until used. Two kinds of manure fibers were used in this study; those collected directly in the farm after separation using a decanter centrifuge - called raw manure fibers - and those which were collected at the Morsø BioEnergi biogas plant after decanting the effluent of the anaerobic digester – called digested manure fibers.

*Analytical methods.* Determination of total solids (TS) and volatile solids (VS) was carried out according to standard methods [14]. Total and soluble Chemical Oxygen Demand (COD) were measured with Hach Lange kits LCK-914 and LCK-514 respectively. Ammonium nitrogen (NH<sub>4</sub>-N) analysis was performed with Hach Lange kit LCK-305. Phosphorus analysis was carried out by applying persulphate digestion and subsequent ascorbic acid photometric determination according to standard methods [14]. Detection and quantification of sugar monomers (glucose, xylose and arabinose) was made with HPLC-RI equipped with an Aminex HPX-87H column (BioRad) at 60°C. Two groups of carbohydrates were determined in the samples of raw and pretreated manure fibers: total carbohydrates, including those bound in the lignocellulosic biomass and simple sugars [15]. Analysis of the two groups of carbohydrates was carried out based on the NREL analytical procedures [16]. Biogas composition in methane was measured with a gas chromatograph (SRI GC model 310) equipped with a thermal conductivity detector and a packed column (Porapak-Q, length 6ft and inner diameter 2.1 mm).

*Effect of AAS on the composition of raw and digested manure fibers.* Samples of manure fibers were soaked in ammonia reagent (32% w/w in ammonia) with a ratio of 10 mL reagent per 1 g TS. Raw and digested manure fibers were subjected to AAS pretreatment for 3 days at 22°C (according to Jurado et al. [13]). Ammonia distillation was performed using a rotary evaporator (Buchi RII Rotavapor) and the composition of fibers was determined before and after the pretreatment.

*Effect of organic loading of AAS pre-treated manure fibers on methane production.* Methane potential tests of pretreated fibers (AAS-fibers) were carried out at triplicates in sealed serum vials and anaerobic mixed liquor from a mesophilic digester treating liquid manure was used as inoculum. Control triplicates were run in parallel by using non-pretreated fibers (control-fibers) at the same TS loadings as previously mentioned while a triplicate containing only inoculum was served as control for all vials. The vials were incubated under mesophilic conditions (37°C) for 35-50 days with periodic shaking and methane production was monitored throughout the duration of the experiments. Methane potential was calculated as the volume of methane produced per g of TS of manure fibers added after subtracting the methane produced in the control vials with only inoculum added.

## 3- RESULTS AND DISCUSSION

*Effect of AAS on the composition of manure fibers.* The composition of raw and digested manure fibers before and after AAS pretreatment is shown in table 1. The TS content of raw and digested fibers was  $31.98 \pm 0.2\%$  and  $27.82 \pm 0.98\%$ , respectively. Soluble COD values imply that solubilisation of the solid matrix took place with AAS pretreatment (6.6% and 14.7% for digested fibers and 5.8% and 8.1% for raw fibers before and after AAS pretreatment, respectively). Solubilisation was more evident in digested than in raw fibers.

	Digested fibers		Raw fibers	
	Control-fibers	AAS-fibers	Control-fibers	AAS-fibers
Glucan, g (100g TS) <sup>-1</sup>	11.8 ±0.1	10.9±0.2	18.6 ±0.6	19.5 ±0.8
Xylan, g (100g TS) <sup>-1</sup>	10.8±0.1	11.6 ±0.0	14.2 ±0.4	13.0 ±0.3
Arabinan, g (100g TS) <sup>-1</sup>	3.7 ±0.1	3.4 ±0.1	7.3 ±0.1	7.3 ±0.3
Klason Lignin, g (100g TS) <sup>-1</sup>	26.8 ±4.3	17.5 ±5.0	17.1 ±1.2	16.8 ±0.7
Free glucose, g (100g TS) <sup>-1</sup>	<sup>b</sup>	0.1 ±0.0	<sup>b</sup>	0.1 ±0.0
Free xylose, g (100g TS) <sup>-1</sup>	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>
Free arabinose, g (100g TS) <sup>-1</sup>	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>	<sup>b</sup>
Soluble COD, g (100g TS) <sup>-1</sup>	6.6 ±0.4	14.7 ±1.4	5.8 ±0.1	8.1 ±0.2
Total COD, g (100g TS) <sup>-1</sup>	97.9±4.7	99. ±6.1	115.0 ±5.9	106.7 ±10.2
NH <sub>4</sub> -N, g (100g TS) <sup>-1</sup>	0.6 ±0.66	1.1 ±0.1	0.96 ±0.2	0.12 ±0.0

<sup>b</sup> below detectable levels

Table 1. Composition of control-fibers and AAS-fibers.

As it was expected and according to the literature [5] cellulose (glucan fraction) was not degraded during the pretreatment accounting approximately for the 12 and 18% of TS in digested and raw fibers respectively. Xylan (corresponding to hemicelluloses fraction) did not seem to be degraded either accounting approximately for the 11 and 14% of TS in digested and raw fibers respectively. The decreased concentration of glucan and xylan in digested fibers compared to the raw fibers could be attributed to the fact that digested fibers had already undergone an anaerobic digestion step resulting thus to a small decomposition of the carbohydrate fractions. In order to verify that AAS did not result in solubilisation of sugars, free sugars were determined as well before and after AAS treatment and in all cases they remained below detection limit (Table 1).

Ammonia concentration differed slightly among different types of fibers and in all cases the amount of ammonia was low enough (0.6-1.1 g NH<sub>4</sub>-N/L for digested fibers and 0.77-0.16 g NH<sub>4</sub>-N/L for raw fibers) to allow anaerobic digestion to proceed without inhibition. According to the literature, unadapted microorganisms can tolerate ammonia concentration up to 1.5-2.5 g NH<sub>4</sub>-N/L before inhibition is observed [17]. Moreover, the methanogenic inoculum used was adapted to an ammonia-N concentration of around 4 g/L.

*Effect of organic loading of AAS pre-treated manure fibers on methane production.* Different organic loadings of AAS pretreated raw and digested fibers (i.e. 0.16, 0.25, 0.5 and 1 g TS per 10 ml of inoculum) were tested in order to analyse possible inhibition due to components that may be formed during the pretreatment.

Duration (d)	10				20				40			
Loading (g-TS/10 ml)	0.16	0.25	0.5	1	0.16	0.25	0.5	1	0.16	0.25	0.5	1
Control-fibers (ml CH <sub>4</sub> /g-TS)	57	58	54	56	87	86	81	85	88	90	84	100
AAS-fibers (ml CH <sub>4</sub> /g-TS)	84	91	85	61	113	113	115	114	112	120	116	121

Table 2. Methane yield after 10, 20 and 40 days of anaerobic digestion of control-fibers and AAS-fibers at different loadings (g TS per 10 ml of inoculum) for digested fibers.

The final methane yields of non-pretreated (control fibers) and AAS-pretreated (AAS-fibers) digested fibers after 40 days of batch anaerobic digestion at different organic loadings are shown in table 2. It is obvious that increasing of TS loading up to 1 g TS per 10 ml of inoculum did not affect the final methane yield of either control- or AAS-fibers. Methane

yield of control-fibers was around 85 ml CH<sub>4</sub>/g TS for all loadings while the final methane yield of AAS-fibers was approximately 120 ml CH<sub>4</sub>/g TS again for all loadings.

In case of AAS-fibers, even though the final methane yield was the same for all loadings tested, the methane production rate (by comparison of CH<sub>4</sub> production in the different time intervals) was different (table 2). Specifically, during the first 10 days, the methane production in the vials with 0.5 and 1 g TS per 10 ml of inoculum was lower than the production observed for the first two loadings (0.16 and 0.25 g TS per 10 ml of inoculum) with the production decreasing with increasing loading. This implies that an inhibition, most probably due to inhibitors formed during the pretreatment, occurred. The inhibition was overcome after 20 days of digestion where the methane production reached the same level for all loadings. Apparently, the microbial culture could quickly adapt and this is an indication that inhibition will not constitute a problem in a continuous process for anaerobic digestion of AAS pretreated digested fibers, at least up to the loadings tested.

The final methane yields of non-pretreated (control fibers) and AAS-pretreated (AAS-fibers) raw fibers after approximately 40 days of batch anaerobic digestion may be seen in table 3. Increment in TS loading from 0.16 to 1 g TS per 10 ml of inoculum did not affect the methane yield of control-fibers. Methane yield was around 115 ml CH<sub>4</sub>/g TS for all loadings. On the other hand, the same increment in TS loading of AAS-fibers resulted in reduced methane yield for loadings higher than 0.16 g TS / 10 ml of inoculum. Specifically, the vials loaded with 0.16 gTS/10 ml of inoculum exhibited a methane yield of 320 ml CH<sub>4</sub>/g TS while the methane yield decreased to 180 ml CH<sub>4</sub>/g TS for the vials loaded with 0.25 and 0.5 gTS/10 ml inoculum. The methane yield became even lower (139 ml CH<sub>4</sub>/g TS) in the vials loaded with the higher 1 gTS/10 ml of inoculum (table 3). This implies the presence of methanogens' inhibitors possibly due to the AAS pretreatment of raw fibers. Furthermore, the inhibitory effect could not be overcome with time (at least within 40 days), contrary to what happened with AAS-pretreated digested fibers.

<b>Duration (d)</b>	<b>13</b>				<b>23</b>				<b>40</b>			
<b>Loading (g-TS/10 ml)</b>	0.16	0.25	0.5	1	0.16	0.25	0.5	1	0.16	0.25	0.5	1
<b>Control-fibers</b> (ml CH <sub>4</sub> /g-TS)	83	89	81	108	100	114	100	121	115	114	107	129
<b>AAS-fibers</b> (ml CH <sub>4</sub> /g-TS)	260	159	166	34	305	182	187	105	320	180	184	139

Table 3. Methane yield after 13, 23 and 40 days of anaerobic digestion of control-fibers and AAS-fibers at different loadings (g TS per 10 ml of inoculum) for raw fibers.

The methane production rate of control-fibers (table 3) was also the same for all loadings tested. However, the methane production rate was significantly reduced in the vials with a loading of 0.25, 0.5 and 1 g TS AAS-fibers / 10 ml of inoculum (table 3) compared to the vials with the lowest loading of 0.16. An increase of the methane production was observed for the highest loading between 13 and 23 days of digestion; however the methane production reached a significantly lower level than in the vials with the lowest loading. The results obtained clearly imply that a strong inhibition of methanogenesis occurred with AAS-pretreated raw fibers at increased loadings. It is likely that this inhibition will be overcome in a continuous system where the microbial community will be exposed for long enough time to adapt to the inhibitors. However, this hypothesis needs to be further investigated.



#### 4- CONCLUSIONS

In the present study, aqueous ammonia soaking (AAS) was successfully applied as a pretreatment method to increase methane potential of swine manure fibers. Specifically, AAS resulted at a 40-80% and 170% increase in methane yield from digested and raw manure fibers, respectively. Batch anaerobic digestion of AAS-treated digested manure fibers could stand loadings as high as 100 g TS / L inoculum with no inhibition problems.

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