Downloaded from orbit.dtu.dk on: Apr 15, 2024

DTU Library

Effect of Aqueous Ammonia Soaking on the Methane Yield and Composition of Digested Manure Fibers Applying Different Ammonia Concentrations and Treatment Durations

Mirtsou-Xanthopoulou, Chrysoula; Jurado, Esperanza; V. Skiadas, Ioannis; Gavala, Hariklia N.

Published in:
Energies

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Mirtsou-Xanthopoulou, C., Jurado, E., V. Skiadas, I., & Gavala, H. N. (2014). 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.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Energies **2014**, *7*, 4157-4168; doi:10.3390/en7074157

OPEN ACCESS

energies

ISSN 1996-1073

www.mdpi.com/journal/energies

Article

Effect of Aqueous Ammonia Soaking on the Methane Yield and Composition of Digested Manure Fibers Applying Different Ammonia Concentrations and Treatment Durations

Chrysoula Mirtsou-Xanthopoulou, Esperanza Jurado, Ioannis V. Skiadas and Hariklia N. Gavala *

Section for Sustainable Biotechnology, Chemistry and Environmental Engineering, Department of Biotechnology, Aalborg University, Copenhagen (AAU-Cph), A C Meyers Vænge 15, DK 2450 Copenhagen, Denmark; E-Mails: chrymx@gmail.com (C.M.-X.); eje@bio.aau.dk (E.J.); ivs@bio.aau.dk (I.V.S.)

* Author to whom correspondence should be addressed; E-Mail: hng@bio.aau.dk or hari_gavala@yahoo.com; Tel.: +45-9940-2586; Fax: +45-9940-2594.

Received: 12 May 2014; in revised form: 19 June 2014 / Accepted: 20 June 2014 / Published: 30 June 2014

Abstract: The continuously increasing demand for renewable energy sources renders anaerobic digestion one of the most promising technologies for renewable energy production. Due to the animal production intensification, manure is being used as the primary feedstock for most biogas plants. Thus, their economical profitable operation relies on increasing the methane yield from manure, and especially of its solid fraction which is not so easily degradable. In the present study, aqueous ammonia soaking (AAS) at six different concentrations in ammonia (5%, 10%, 15%, 20%, 25% and 32%) and for 1, 3 and 5 days at 22 °C was applied on digested fibers separated from the effluent of a manure-fed, full-scale anaerobic digester. A methane yield increase from 76% to 104% was achieved during the first series of experiments, while the difference in reagent concentration did not considerably affect the methane yield. It was shown that the optimal duration was three days for both 5% and 25% w/w reagent concentrations in ammonia tested. Carbohydrates and phosphorus content remained unaffected, while a slight decrease in Klason lignin and non-soluble organic nitrogen content was observed after AAS. It is concluded that AAS is a very promising treatment resulting to an overall increase of the methane yield of digested manure fibers from 76% to 265% depending on the conditions and the batch of digested fibers used (an even higher increase of 190%–265% was achieved during the 2nd series of experiments, where different AAS durations were tested, compared to the 1st series were different ammonia concentrations were applied).

Keywords: anaerobic digestion; aqueous ammonia soaking; digested manure fibers; methane potential; pretreatment

1. Introduction

Anaerobic digestion is one of the most promising renewable energy technologies, as it provides a solution to both environmental and energy considerations. Some of the benefits of this technology are that it reduces the odor, reduces the amount of the organic wastes, contributes to the reduction of the Greenhouse Gases (GHG) emissions and produces a high value fertilizer as well as a renewable energy gas [1].

Nowadays, the intensification of animal production generates large amounts of manure, which in Denmark is mainly treated by anaerobic digestion. Increasing the methane productivity of manure has become a necessity for biogas plants to be economically viable. The addition of biomass products with high methane yield, e.g., fat sludge or fish oil [2] and the introduction of solid-liquid separation technologies [3] are some of the strategies that have been adopted the last years in order to increase the methane yield of manure. However, digestion of manure with other biomass products is not always feasible and economically attractive as the volumes of such wastes streams are limited.

Thus, separation of the liquid-solid fraction of the manure has been lately in the forefront. In this way, the liquid part of the effluent, which contains most of the water, the minerals and part of the carbon of the influent, can be effectively used as a fertilizer in the farms and the solid fraction, called as manure fibers, could be transported to centralized biogas plants using the existing infrastructure for solids transportation [4]. However, manure fibers contain mainly lignocellulosic plant fibers and swine faeces as well as some hair, skin and soil from roughage and they are characterized by low methane yield due to low biodegradability of their main components [4]. Therefore, a pre- or post-treatment (before and after anaerobic digestion, respectively) is necessary in order to increase their biodegradability and methane yield.

Post-treatment of already digested fibers, for being recirculated to the digester, presents several advantages over pre-treatment of raw fibers (coming after the separation of the manure in liquid and solid fraction). Post-treatment of digested fibers focuses only on hardly biodegradable biomass compared to raw fibers where easily biodegradable matter is also present. Therefore, the mass of fibers to be treated is expected to be significantly lower in case of digested fibers leading thus to a more economical process. Additionally, higher organic loadings [5] of the pre-treated raw fibers are more likely to cause inhibition during the anaerobic digestion process.

The biodegradability problem of manure and especially of the fibers has captured the attention of many researchers. Different kinds of pretreatments have been applied so far to overcome the low digestibility problems. The main goal of all pretreatments is to increase the accessible surface area for the enzymes so the hydrolysis step can occur at a higher rate [6]. A successful pretreatment of a lignocellulosic biomass should be able to increase the production of highly digestible solids which will subsequently increase the rate of sugars formation and/or yield. Additionally, it should alleviate the production of inhibitors, avoid the degradation of sugars and recover the highest possible amount of lignin [6]. All these should be carried out through a cost effective and sustainable process by means of energy and power considerations [6]. Some representative pretreatments applied on fibers to enhance the methane productivity are mechanical maceration [7], hydrothermal, chemical, enzymatic [8], steam pretreatment with NaOH [9] as well as wet explosion [10].

A chemical pretreatment, that was investigated in the past for increasing the digestibility of ruminants feed [11,12] and that has been applied lately to different lignocellulosic biomasses mainly for bioethanol production, is aqueous ammonia soaking (AAS). Some biomasses tested so far against this pretreatment are barley hull [13], corn stover [14] as well as rice straw [15]. Ammonia, which is usually the main reagent used for this pretreatment, is a weak base which has a high selectivity towards reactions with lignin [16] preventing in that way any unfavorable loss of sugars. Furthermore, due its high volatility, it can be easily recovered and recycled [17]. Thus, there is no need for further chemicals consumption offering a more cost effective and sustainable way of treatment. Additionally and compared to other chemicals, ammonia is quite safe to handle, it is non-polluting and non-corrosive [17] and it can be used both in high and low temperatures. However, lower temperatures are more favored as they can alleviate problems such as a higher energy input, loss of sugars or the formation of toxic compounds [17]. Recently, AAS has been applied on switchgrass [18,19], wheat straw, miscanthus and willow [20] for enhancing the methane yield. Also, it has been proved that it is a very efficient method for increasing the methane yield from raw manure fibers [5] while application of AAS on manure fibers in biogas plants already equipped with ammonia removal infrastructure (as ammonia removal is in most cases necessary for manure based plants) could constitute a cost-efficient and sustainable pretreatment (or post-treatment) option.

In cases where the digested manure fibers will be further utilized as a fertilizer in the farms, the effect of a pre- or post-treatment on nutrients concentration and form should also be tested. Depending on the nutrition of the animal, manure may contain nutrients concentrations important for further crop production [21]. Thus, many methods have been developed for achieving an efficient removal of some vital nutrients, such as nitrogen and phosphorus from the effluent of a biogas plant [22] and the effect of a pre- or post-treatment on the available nutrients is considered of vital importance.

In the present study, aqueous ammonia soaking (AAS) has been applied to digested fibers in order to increase the methane yield. AAS treatment was performed under six different reagent concentrations (5%, 10%, 15%, 20%, 25%, 32%) and three different duration times (1, 3, and 5 days) in order to assess the effect that ammonia concentration and treatment duration have on the efficiency of the treatment in terms of methane yield increase. The effect of the AAS treatment on the composition of the digested fibers was also investigated, through measurements of total carbohydrates, Klason lignin and of different forms of nitrogen and phosphorus.

2. Materials and Methods

2.1. Substrate and Inoculum

Digested manure fibers were kindly provided by Morsø BioEnergi (Redsted Mors, Denmark, a mesophilic biogas plant treating manure and manure fibers) and stored at −20 °C until used.

The inoculum for the methane potential tests came from a 3-L active volume mesophilic digester treating swine manure at an organic loading rate of 2.83–3.53 g·COD·L^{-1}·d^{-1} (chemical oxygen demand) and a methane productivity of 0.57 ± 0.08 L·L^{-1}·d^{-1} and it was characterized by a 17.6 ± 1.4 g·L^{-1} volatile solids (VS) content.

2.2. Analytical Methods

All characterizations of AAS-treated fibers were done after removal of ammonia (as described in paragraph 2.3). The results are given per g total solids (TS) in order to be comparable with those coming from non-treated fibers as the mass of TS before and after AAS treatment remained the same. Total (TS) and volatile (VS) solids were measured according to standard methods [23]. For total phosphorus and total nitrogen determination, material was dried at 42 °C overnight and powdered, while for the soluble and inorganic measurements, material was centrifuged at 10,000 rpm for 10 min and the supernatant was passed through 0.2 μm membrane filters. For the organic forms of phosphorus persulphate digestion was applied followed by the ascorbic acid photometric method. While for the organic forms of nitrogen, digestion with the micro-Kjeldahl apparatus followed by titration (titrimetric method) was applied as well according to standards methods [23].

For the determination of total carbohydrates and Klason lignin, the material was dried at 42 °C overnight and powdered, while for the determination of free sugars the material was centrifuged at 1000 rpm and the supernatant was passed through 0.2 μm size membrane filters. Analysis of these groups of sugars was performed according to the NREL (National Renewable Energy Laboratory) analytical procedures [24]. Prior to the High Performance Liquid Chromatography (HPLC) analysis samples were first centrifuged at 10,000 rpm for 10 min, and after being filtered with 0.45 μm membrane filters, H_2SO_4 was added to adjust the pH between 2 and 3. Detection and analysis of all these sugar monomers (glucose, xylose, arabinose) was performed by a High Performance Liquid Chromatography. The HPLC system was a Dionex Ultimate 3000 model (Germering, Germany) consisted of a BioRad (Hercules, CA, USA) Aminex HPX-87H ion exclusion column and a RIc (refractive index) detector. The temperature of the column was 60 °C, while the eluent was 4 mM sulfuric acid (H_2SO_4) with a flow rate of 0.6 mL·min^{-1}. The injection volume was 10 μL.

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 6 ft and inner diameter 2.1 mm). The temperature for injector, column and detector was set to 80 °C. The volume of methane produced in sealed vials during methane potential tests was calculated by multiplying the biogas composition in methane with the headspace volume. The increase in methane yield of the AAS-treated fibers compared to the non-treated fibers was calculated according to Equation (1):

$$\%\ \text{increase} = \frac{Y_{\mathrm{CH_4}}^{\text{AAS-fibers}} - Y_{\mathrm{CH_4}}^{\text{control-fibers}}}{Y_{\mathrm{CH_4}}^{\text{control-fibers}}} \times 100 \quad (1)$$

Where $Y_{\mathrm{CH_4}}^{\text{AAS-fibers}}$ and $Y_{\mathrm{CH_4}}^{\text{control-fibers}}$ is the methane yield in mL CH_4 g^{-1} TS obtained from AAS-treated fibers and non-treated fibers, respectively. The mass of TS used for the calculations was the mass of TS that was added in each test. Thus, any dilution during the AAS treatment and ammonia removal did not affect the final result. It is important to mention that there was no loss of TS during AAS treatment.

2.3. Ammonia Pretreatment

Samples of digested manure fibers were soaked in ammonia reagent (with different concentrations in ammonia as specified in Sections 2.4 and 2.5) at a ratio of 10 mL reagent per g TS. The treatment was performed in closed glass flasks to avoid ammonia evaporation. After the completion of the treatment, water was added at a ratio of 10 mL per g TS to facilitate the subsequent ammonia distillation step. Distillation was performed using a rotary evaporator (RII Rotavapor, Buchi, Flawil, Switzerland) with a vertical condenser.

2.4. Effect of Different Reagent Concentration in Ammonia on the Methane Production from AAS Treated Digested Fibers

Methane potential tests were performed in triplicates in 117 mL sealed serum vials. The vials were initially flashed with a N_2:CO_2 gas mixture to ensure anaerobic conditions and subsequently supplied with an amount equivalent to 0.5 g TS of AAS-treated digested fibers and 40 mL inoculum. In total, 6 experimental triplicate vials were prepared with digested fibers been treated with different reagent concentration in ammonia, namely, 5%, 10%, 15%, 20%, 25% and 32% w/w, for three days at 22 °C. Triplicates with only inoculum as well as triplicates with non AAS-treated digested fibers and inoculum, at the same ratio mentioned above for comparison purposes, served as controls. All vials were incubated at 37 °C for approximately 55 days and methane production was monitored *versus* time.

2.5. Effect of AAS Treatment Duration on the Methane Production from AAS Treated Digested Fibers

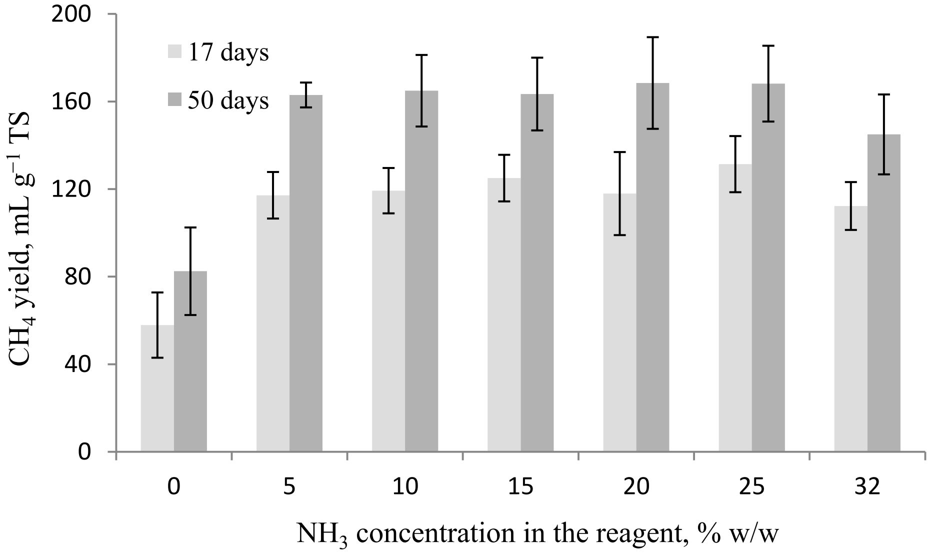
The effect of 1, 3, and 5 days AAS treatment on methane production from digested fibers was investigated in a second batch series. 5% and 25% w/w reagent concentrations in ammonia were applied for AAS treatment since those conditions were identified as the most efficient from the first batch series. Therefore, six experimental triplicates with 0.5 g TS of AAS-treated digested fibers and 40 mL inoculum were prepared as described above with digested fibers having been treated with 5% and 25% ammonia reagent for a period of 1, 3 and 5 days. Triplicates contained only inoculum as well as triplicates with non AAS-treated digested fibers and inoculum, at the same ration mentioned above, served as controls and all vials were incubated at 37 °C for approximately 55 days. Methane production was monitored *versus* time.

3. Results and Discussion

3.1. Effect of Different Reagent Concentration in Ammonia on the Methane Production from AAS Treated Digested Fibers

AAS was applied successfully on digested fibers to increase the methane yield. Soaking in six different reagent concentrations in ammonia (5%, 10%, 15%, 20%, 25%, 32%) was applied for 3 days at 22 °C. The methane yields after 17 and 50 (ultimate methane yields) days of digestion in all vials are shown in Figure 1, in mL CH_4 per g TS.

Figure 1. Methane yield after 17 and 50 days of anaerobic digestion of non-treated (0% in NH_3) and AAS-treated for 3-d digested swine manure fibers with different reagent concentrations in ammonia.



As it can be seen, after the first 17 days, an overall methane yield increase from 93% to 127% was achieved compared to controls (digested manure fibers where AAS was not applied). While after 50 days of digestion the methane yield increase ranged from 76% to 104% implying that AAS positively affected both methane production rate and methane yield. In all cases (except of the AAS with the 32% in ammonia, where the methane yield was slightly lower), methane production increased from about 58 mL·g^{-1} TS to around 120 mL·g^{-1} TS, during the first 17 days. It is remarkable that the reagent concentration at the range tested did not affect significantly the overall methane yield. The lowest reagent concentration used (5%) gave as high yield increase (98%) as almost the 25% reagent concentration (104%). This can be also clearly seen by the ultimate methane yields achieved (after 50 days), where all reagent concentrations (except the 32%) gave almost the same methane yield, around 165 mL·g^{-1} TS methane. Thus, it can be concluded that an ammonia concentration as low as 5% is adequate for achieving the same increase in methane yield as the highest concentrations tested resulting at an even lower cost for recovery and recycling of ammonia in full-scale. Optimization studies extended also to lower ammonia concentrations is necessary as it is anticipated that there is a threshold below of which the effect of ammonia becomes negligible.

3.2. Effect of AAS Treatment on Klason Lignin, Sugars and Nutrients (N, P) Concentration and Forms

Characterization of digested fibers before and after AAS treatment was conducted in terms of total solids, total carbohydrates and Klason lignin as well as of the different forms of nitrogen and phosphorus. The results obtained can be seen in Table 1. The TS content of the non-pretreated digested fibers was 28.86 ± 0.9 g TS 100 g^{-1} material, while the average TS content for the AAS pretreated digested fibers was 4.15 ± 0.15 g TS 100 g^{-1} material with a TS VS^{-1} ratio equal to 1.44. The difference in TS concentration was due to the dilution during the AAS and ammonia removal processes described in Section 2.3.

Table 1. Characterization results of digested fibers before and after 3-d AAS treatment.

Characteristics ($g\ 100\ g^{-1}$ TS)	No AAS	AAS (5%)	AAS (10%)	AAS (15%)	AAS (20%)	AAS (25%)	AAS (32%)
Glucan	17.1 ± 1.8	18.4 ± 0.2	21.1 ± 1.2	18.7 ± 0.3	22.9 ± 1.4	14.9 ± 1.7	19.14 ± 0.06
Xylan	12.74 ± 1.16	12.4 ± 0.0	13.4 ± 0.7	10.97 ± 0.5	14.1 ± 0.9	9.1 ± 0.8	8.27 ± 0.01
Arabinan	5.11 ± 0.03	4.01 ± 0.0	4.7 ± 0.3	3.99 ± 0.3	4.6 ± 0.1	3.8 ± 0.1	3.29 ± 0.00
Klason Lignin	24.88 ± 1.17	21.6 ± 0.4	21.5 ± 0.6	22.3 ± 1.1	23.2 ± 1.3	24.2 ± 0.8	24.70 ± 0.73
Free Sugars	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Non Soluble organic N	1.74 ± 0.25	1.72 ± 0.92	1.07 ± 0.07	1.39 ± 0.09	1.61 ± 0.11	1.62 ± 0.22	2.11 ± 0.21
Soluble organic N	0.6 ± 0.02	0.92 ± 0.04	1.07 ± 0.01	0.99 ± 0.03	0.98 ± 0.05	0.98 ± 0.04	0.73 ± 0.02
Inorganic N	0.29 ± 0.03	0.83 ± 0.02	0.85 ± 0.01	0.81 ± 0.02	0.72 ± 0.03	0.73 ± 0.02	0.63 ± 0.03
Non Soluble organic P	2.05 ± 0.2	2.12 ± 0.15	2.62 ± 0.01	2.46 ± 0.21	2.02 ± 0.13	1.77 ± 0.18	2.35 ± 0.29
Soluble organic P	0.03 ± 0.0	0.1 ± 0.0	0.07 ± 0.0	0.14 ± 0.0	0.13 ± 0.01	0.18 ± 0.04	0.12 ± 0.02
Inorganic P	0.098 ± 0.01	0.08 ± 0.01	0.04 ± 0.0	0.11 ± 0.01	0.06 ± 0.00	0.15 ± 0.03	0.07 ± 0.002

[a] Below detectable levels.

Results shown in Table 1 are in agreement with the study of Kim *et al.* [14] where pre-treatment did not have a great impact on the total carbohydrates content. Glucan, xylan and arabinan accounted for the 17.1 ± 1.8, 12.7 ± 1.2 and 5.1 ± 0.03 percent (%) of the total solids content of digested fibers, respectively. While for the AAS fibers the content of glycan, xylan and arabinan had an average value of 19.2 ± 0.8, 11.4 ± 0.5 and 4.1 ± 0.1 percent (%) of the TS content, respectively. Similar results had been obtained by Gupta and Lee [16] where AAS (different modifications of AAS treatment) was applied on switch grass. Last but not least, recent studies have shown that alkaline treatments may cause some transformation of sugars to carboxylic acids such as acetate [8]. A slight increase in acetate content had also been observed in the present study, where the concentration of acetate increased from 1.1 $g{\cdot}L^{-1}$ to 1.4 $g{\cdot}L^{-1}$ when the digested fibers treated with the highest reagent concentration in ammonia (32%). In overall we could say that ammonia did not cause any remarkable degradation of sugars, thus fulfilling one of the goals (mentioned above) that a successful pre-treatment should achieve.

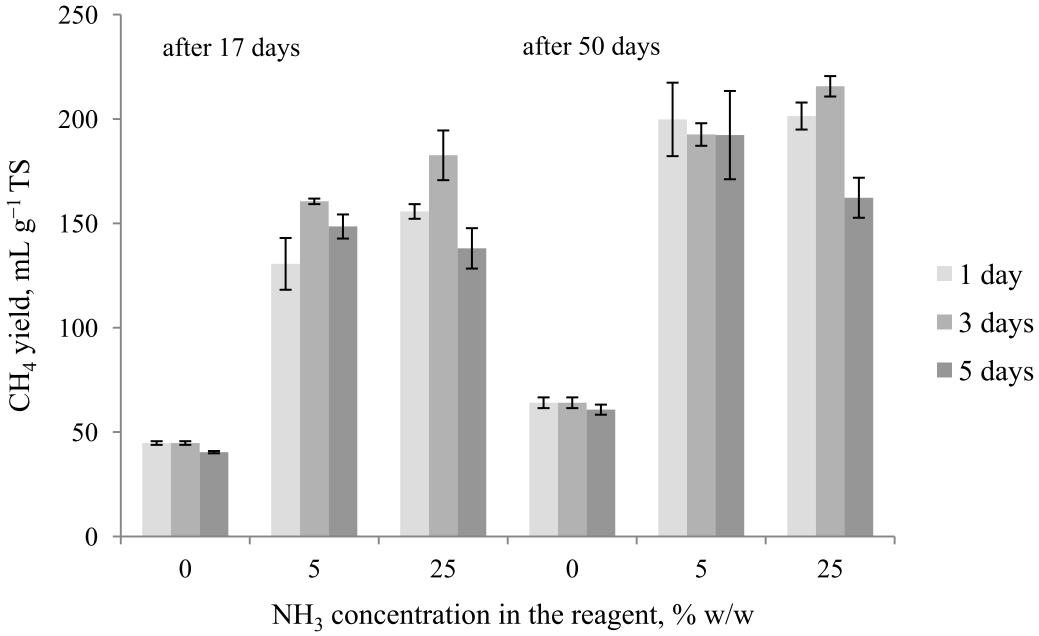
Klason lignin content was 24.9 g per 100 g TS of the digested fibers while for the AAS pretreated ones it was lying between 24.7 and 21.5 g per 100 g TS. Kim *et al.* [17] stated characteristically that ammonia is a proven delignification reagent. What is interesting as well is that generally the lower reagent concentrations resulted in a higher delignification degree. In overall, we can say that there is an increasing, albeit slight, reduction in the Klason lignin content with decreasing reagent concentration in ammonia.

In the current study, non-soluble organic, soluble organic and inorganic phosphorus and nitrogen have also been measured (Table 1) in order to assess the effect of AAS pretreatment on the concentration and the different forms of nitrogen and phosphorus. The total nitrogen and phosphorus content remained the same. An increasing tendency was observed in the soluble organic nitrogen content of AAS-treated fibers with 10%–32% NH_3 in the reagent. That increase, combined with the corresponding decrease of the non-soluble nitrogen content could be attributed to a slight solubilization of proteins due to the AAS treatment. On the other hand, the different phosphorus forms remained unaffected by the AAS treatment with average values of 2.20 ± 0.27, 0.11 ± 0.04 and 0.09 ± 0.03 g-P per 100 g TS for non-soluble organic, soluble organic and inorganic phosphorus, respectively.

3.3. Effect of AAS Treatment Duration on the Methane Production from AAS Treated Digested Fibers

The effect of 1, 3, and 5 days AAS treatment on methane production from digested fibers was investigated in a second batch series. As the 5% and 25% w/w reagent concentration in ammonia were identified as the most efficient regarding methane yield from the first batch series, they were chosen to be applied in the second batch series where the effect of AAS duration on methane yield from digested manure fibers was investigated. Therefore, three different duration periods (1, 3 and 5 days) were applied for the two (5% and 25% w/w in ammonia) reagent concentrations. Methane yield after 17 and 55 days of anaerobic digestion are shown in Figure 2.

Figure 2. Methane yield after 17 and 50 days of anaerobic digestion of non-treated and AAS-treated digested swine manure fibers with 5% and 25% reagent concentrations in ammonia.



As one can see from Figure 2, reagent concentration of 5% w/w in ammonia applied for 1, 3 and 5 days increased the ultimate CH_4 yields (50 days) from 64 mL·g^{-1} TS to 199, 192 and 192 mL·g^{-1} TS, while in the case of 17 days of digestion the yield increased from 44 mL·g^{-1} TS to 130, 160 and 148 mL·g^{-1} TS, respectively. In both cases, the methane yield increase, compared to the controls, was more than 200%. Similar results obtained with the 25% w/w in ammonia reagent concentration, where the CH_4 yields for 1, 3 and 5 days AAS duration, increased up to 201, 215 and 162 mL·g^{-1} TS after 50 days of digestion, and up to 155, 182, and 138 mL·g^{-1} TS after 17 days of digestion, respectively. The average percentage increase in the methane yield was around 205% for the 50 days, and around 265% for the 17 days. It seems that the optimal duration among the ones tested is the three days for both reagent concentrations. One might also conclude that the optimal AAS duration is independent on the reagent concentration. However, optimization studies extended also to lower durations in combination with techno economic studies of the process is the next step to be done for drawing more solid conclusions. It is also noticeable that the increase in methane was higher than the increase obtained from the first batch series. This was attributed to the fact that the first batch series was performed with a different batch of digested fibers, with a 23% higher content in Klason lignin than the second batch (24.9 compared to 20.2 g 100 g^{-1} TS). The possibility for increasing the methane yield of manure fibers has so far been investigated through different kind of methods, which are summarised in Table 2.

Table 2. Treatments applied on manure fibers and the corresponding maximum methane yield increase.

Pretreatment Conditions	Substrate	Highest CH_4 yield increase (%)	Reference
Chemical treatment with Steam and H_2SO_4 (2.1% w/w)	Cow and pig manure digested fibers	67	[9]
Chemical treatment with CaO	Cow and pig manure digested fibers	66	[8]
Physical treatment (size reduction)	Cow and pig manure digested fibers	10	[8]
Steam treatment with NaOH	Cow and pig manure digested fibers	26	[8]
Steam + Laccase + H_3PO_4	Cow and pig manure digested fibers	18	[8]
Steam + Laccase + NaOH	Cow and pig manure digested fibers	34	[8]
Mechanical maceration	Cattle Manure fibers	20	[25]
Biological treatment with B4 hemicellulose bacterium	Cattle Manure fibers	30	[25]
Chemical treatment with NaOH	Cattle Manure fibers	23	[25]
Chemical treatment with NH_4OH	Cattle Manure Fibers	23	[25]
Chemical treatment NaOH:KOH:$Ca(OH)_2$	Cattle Manure Fibers	20	[25]
Mechanical maceration	Manure Fibers	25	[7]
Wet explosion	Digested manure fibers	136	[10]
Aqueous ammonia soaking	Raw swine manure fibers	170	[5]
Aqueous ammonia soaking	Digested swine manure fibers	265	Present study

According to Angelidaki and Ahring [25] biological treatment of fibers with a hemicellulose degrading bacterium resulted in a 30% methane increase, while according to Bruni *et al.* [9], physicochemical treatment using steam, high temperature and sulphuric acid resulted to a 67% methane increase compared to the untreated fibers. In the study of Biswas *et al.* [10] an even higher increase of 136% has been reported based on a wet explosion method. In fact, the latter is among the higher increases of methane yield of digested manure fibers that have been reported so far in the international literature. Aqueous Ammonia Soaking resulted in comparable and even higher increases in methane yield (as derived from [5] and the present study) with the additional advantages of no chemicals and high energy consumption, rendering it in one of the most sustainable and economically viable ways of treatment tested so far on manure fibers. A costs and benefits analysis of the AAS treatment combined with an ammonia recovery method applicable in biogas plants is part of future research plans.

4. Conclusions

In the current study, Aqueous Ammonia Soaking was successfully applied as a method to increase the methane potential of digested manure fibers. Soaking in six different reagent concentrations in ammonia (5%, 10%, 15%, 20%, 25%, 32%) was applied for 3 days at 22 °C. An overall methane yield increase from 85% to 110% was achieved compared to controls (digested manure fibers where AAS was not applied). The difference in reagent concentration at the range of 5%–25% w/w in ammonia did not affect that much the overall methane yield resulting to an increase of 76%–104% compared to the non AAS-treated fibers. Thus, an ammonia concentration as low as 5% is adequate for achieving the same increase in methane yield as the highest concentrations tested; it is anticipated that this will result to an even lower cost for recovery and recycling of ammonia in full-scale. Moreover, the effect of 1, 3, and 5 days AAS treatment on methane production from digested fibers was investigated with 5 and 25% w/w reagent concentrations in ammonia. It was shown that the optimal duration among the ones tested was the three days for both reagent concentrations. In overall, an increase of the methane yield of digested manure fibers from 76% to 265% was obtained in the present study depending on the conditions and the batch of digested fibers used. Last but not least, it was found that carbohydrates content remained unaffected, while a slight decrease in Klason lignin content was observed with decreasing reagent concentrations in ammonia. A slight solubilisation of non-soluble organic nitrogen was also noticed while AAS treatment had no effect on the different forms of phosphorus.

Acknowledgments

The authors wish to thank the EUDP-2008, Energistyrelsen, Copenhagen for the financial support of this work under RETROGAS project.

Author Contributions

This paper is part of the Master thesis of Chrysoula Mirtsou-Xanthopoulou who performed all the experiments and wrote the first draft of the manuscript. Esperanza Jurado participated in the planning of the experiments and in the practical guidance and every day supervision of the experimental procedures. Ioannis Skiadas participated in planning the experiment, supervised the setting up of the experimental devices, solved technical problems and revised the manuscript. Hariklia Gavala was the supervisor of the Master project, she had the overall responsibility for organizing and supervising the research work and data processing.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rico, C.; Rico, J.L.; Tejero, I.; Munoz, N.; Gomez, B. Anaerobic digestion of the liquid fraction of dairy manure in pilot plant for biogas production: Residual methane yield of digestate. *Waste Manag.* **2011**, *31*, 2167–2173.

2. Angelidaki, I.; Ellegaard, L. Codigestion of manure and organic wastes in centralized biogas plants. *Appl. Biochem. Biotechnol.* **2003**, *109*, 95–105.
3. Burton, C. The potential contribution of separation technologies to the management of livestock manure. *Livest. Sci.* **2007**, *112*, 208–216.
4. Jurado, E.; Gavala, H.N.; Rohold, L.; Skiadas, I.V. Cost-effective production of biogas from manure—Retrogas project. In Proceedings of the WasteEng10 3rd International Conference on Engineering for Waste and Biomass Valorisation, Beijing, China, 17–19 May 2010; p. 224.
5. Jurado, E.; Skiadas, I.V.; Gavala, H.N. Enhanced methane productivity from manure fibers by aqueous ammonia soaking pretreatment. *Appl. Energy* **2013**, *109*, 104–111.
6. Brodeur, G.G.; Yau, E.E.; Badal, K.K.; Collier, J.J.; Ramachandran, K.B.K.; Ramakrishnan, S.S. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Enzym. Res.* **2011**, *2011*, doi:10.4061/2011/787532.
7. 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.* **2000**, *41*, 145–153.
8. 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.* **2010**, *101*, 8713–8717.
9. Bruni, E.; Jensen, A.P.; Angelidaki, I. Steam treatment of digested biofibers for increasing biogas production. *Bioresour. Technol.* **2010**, *101*, 7668–7671.
10. 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.* **2012**, *66*, 1751–1758.
11. Han, I.K.; Ha, J.K.; Garrett, W.N.; Hinman, N. The Energy Value of Rice Straw for Ruminants as Influenced by Treatment with Anhydrous Ammonia Or Mixing with Alfalfa. *Asian Australas. J. Anim. Sci.* **1989**, *2*, 115–122.
12. Vanabelle, B.; Vandenbroecke, A.; Vanbelle, M. Use of Alkali Treatments in Ruminant Feeding. 2. Ammonia Treatment. *Landbouwtijdschrift Rev. Agric.* **1991**, *44*, 685–694.
13. Kim, T.H.; Taylor, F.; Hicks, K.B. Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. *Bioresour. Technol.* **2008**, *99*, 5694–5702.
14. Kim, T.H.; Lee, Y. Pretreatment of corn stover by soaking in aqueous ammonia. *Appl. Biochem. Biotechnol.* **2005**, *124*, 1119–1131.
15. Ko, J.K.; Bak, J.S.; Jung, M.W.; Lee, H.J.; Choi, I.G.; Kim, T.H.; Kim, K.H. Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresour. Technol.* **2009**, *100*, 4374–4380.
16. Gupta, R.; Lee, Y. Investigation of biomass degradation mechanism in pretreatment of switchgrass by aqueous ammonia and sodium hydroxide. *Bioresour. Technol.* **2010**, *101*, 8185–8191.
17. Kim, T.H.; Gupta, R.; Lee, Y.; Mielenz, J. Pretreatment of biomass by aqueous ammonia for bioethanol production. *Methods Mol. Biol.* **2009**, *581*, 79–91.
18. Himmelsbach, J.N. Aqueous Ammonia Soaking (AAS) as a Biomass Pretreatment Method: Pilot-Scale Study with Switchgrass, Bench-Scale Use with Poplar and Methane Potential from Anaerobic Digestion of Pretreated Switchgrass. Master's Thesis, Iowa State University, Ames, IA, USA, 2009.

19. Himmelsbach, J.N.; Raman, D.R.; Anex, R.P.; Burns, R.T.; Faulhabcr, C.R. Effect of Ammonia Soaking Pretreatment and Enzyme Addition on Biochemical Methane Potential of Switchgrass. *Trans. ASABE* **2010**, *53*, 1921–1927.
20. Jurado, E.; Gavala, H.N.; Skiadas, I.V. Enhancement of methane yield from wheat straw, miscanthus and willow using aqueous ammonia soaking. *Environ. Technol.* **2013**, *34*, 2069–2075.
21. Hjorth, M.; Christensen, K.V.; Christensen, M.L.; Sommer, S.G. Solid–liquid separation of animal slurry in theory and practice. A review. *Agron. Sustain. Dev.* **2009**, *30*, 153–180.
22. Karakashev, D.; Schmidt, J.E.; Angelidaki, I. Innovative process scheme for removal of organic matter, phosphorus and nitrogen from pig manure. *Water Res.* **2008**, *42*, 4083–4090.
23. Standard Methods for the Examination of Water and Wastewater. Available online: http://www.standardmethods.org/ (accessed on 24 June 2014).
24. Sluite, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory (NREL). Available online: http://www.nrel.gov/biomaa/pdfs/42618.pdf (accessed on 20 June 2014).
25. Angelidaki, I.; Ahring, B.K. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Sci. Technol.* **2000**, *41*, 189–194.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).