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Improving the Anaerobic Digestion of Swine Manure through an Optimized Ammonia Treatment: Process Performance, Digestate and Techno-Economic Aspects

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Abstract: Swine manure mono-digestion results in relatively low methane productivity due to the low degradation rate of its solid fraction (manure fibers), and due to the high ammonia and water content. The aqueous ammonia soaking (AAS) pretreatment of manure fibers has been proposed for overcoming these limitations. In this study, continuous anaerobic digestion (AD) of manure mixed with optimally AAS-treated manure fibers was compared to the AD of manure mixed with untreated manure fibers. Due to lab-scale pumping restrictions, the ratio of AAS-optimally treated manure fibers to manure was only 1/3 on a total solids (TS) basis. However, the biogas productivity and methane yield were improved by 17% and 38%, respectively, also confirming the predictions from a simplified 1st order hydrolysis model based on batch experiments. Furthermore, an improved reduction efficiency of major organic components was observed for the digester processing AAS-treated manure fibers compared to the non-treated one (e.g., 42% increased reduction for cellulose fraction). A preliminary techno-economic analysis of the proposed process showed that mixing raw manure with AAS manure fibers in large-scale digesters could result in a 72% increase of revenue compared to the AD of manure mixed with untreated fibers and 135% increase compared to that of solely manure.

Keywords: manure; anaerobic digestion; ammonia pretreatment; manure fibers; techno-economic analysis; CSTR; aqueous ammonia soaking; biogas; methane; continuous anaerobic digestion

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1. Introduction

Swine manure is a major source of environmental pollution, contributing significantly to atmospheric, soil, and waterbodies contamination. In presence of the available nutrients, the inherent microorganisms of manure degrade the organic matter remaining after animal digestion, resulting in a series of degradation products, among them CH_4 and CO_2. The controlled microbial degradation of manure through the anaerobic digestion (AD) process is a commonly applied technology, during which the emissions are captured and can be used in the form of biogas as a renewable energy source. Additionally, the digested manure can be disposed in a safer manner as the C content has been stabilized to a significant extent. In Europe, around 65% of manure is handled in liquid form (slurry), containing a mixture of feces, urine, washing water, and bedding material [1]. As a result, the dry matter content of liquid manure is lower than 10%. This fact, in combination with the low degradation rate of the solid fraction (fiber) of manure and the high ammonia content, results in a poor biogas production and consequently in economically non-feasible AD processes [2–4].

In light of the increasing efforts for production of fuels from renewable sources, a lot of focus has been given lately to manure-based AD processes mainly for boosting the biogas production. In turn, this has given rise to co-digestion practices [5], where manure is enriched with easily degradable materials, e.g., residues from the food industry, household waste or energy crops for improving the biogas production. Nevertheless, this practice results in a high demand and thus in an increase of the price of co-substrate materials [6] and to the land-use competition with the food production sector. Moreover, the availability of these co-substrates is often limited in comparison to the amounts of manure generated [4]. Consequently, lower amounts of manure are treated anaerobically, increasing thus the negative environmental impacts from the disposal of untreated manure.

In order to facilitate the efficient manure management, separation of manure to a fiber and a liquid fraction is implemented in some countries [7]. In Denmark the separation of manure is common, resulting in 90,000 tons of fibers being generated annually [8]. Upon separation, the fiber fraction of manure that contains the accumulated recalcitrant organic content could be further pretreated for enhancing their bioconversion. Subsequently, the pretreated fibers can be co-digested with raw manure to increase the biogas production. This approach permits to increase the dry matter content of liquid manure (with fibers that exhibit an improved digestibility) and to reduce the cost of liquid manure transportation to biogas plants due to its reduced volume.

The pretreatment of manure fibers aims at overcoming the recalcitrance of their lignocellulosic structure that significantly slows down their digestion. A significant research effort is noted currently for identifying efficient pretreatments for improving the AD process of lignocellulosic biomasses. Aqueous ammonia soaking (AAS) is a chemical pretreatment that uses NH_3 at low or ambient temperature, that has so far been successfully implemented for improving the CH_4 yield of several biomasses [9–11], and presents certain characteristics that make it a promising process to be applied on manure fibers. Usually chemical pretreatments are not preferred as they are costly due to the consumption of chemicals. The main advantage of AAS is the possibility of removing and recycling the only chemical used, NH_3, relatively easily due to its high volatility [6,12]. The recovered NH_3 stream can be reused for subsequent AAS pretreatments. In case a surplus of NH_3 is needed for the pretreatment, this can be recovered from the digestate, which presents an increased NH_3 concentration compared to the influent manure due to the mineralization of organic N during AD [13]. This process could be facilitated by using waste heat from combined heat and power (CHP) plants that are often associated to biogas plants. The NH_3 can be recovered by stripping or extraction, technologies that are not extensively used in AD plants; however, some biogas plants digesting manure have already adopted them for NH_3 recovery. The NH_3 removal prior to AD also permits reducing the NH_3 concentration of manure fibers to values lower than initial, alleviating thus manure-based AD from NH_3 stress. Then again, given that ambient temperature and pressure is applied during the pretreatment, low energy input is anticipated. Finally, a recent study showed that swine manure fibers respond greatly to this pretreatment and when applied under optimal conditions, a 244% increase of CH_4 yield can be obtained in batch AD [14]. Batch experiments can be very useful for indicating the biodegradability rate of substrates as well as for a fast comparison of AD under different conditions or substrates. Nevertheless, as the majority of industrial-scale digesters operate in continuous mode, continuous AD experiments can provide valuable information on the performance of a process that is closer to a real application [15].

Following the efficiency of AAS on swine manure fibers demonstrated up to now from batch experiments and due to the promising characteristics of this pretreatment, the present work aims at assessing the efficiency of continuous AD of manure enriched with optimally AAS-treated fibers, as compared with the efficiency of continuous AD of manure enriched with untreated fibers. Focus was given on the biogas and CH_4 productivity of the digesters as well as on the reduction efficiency of the different organic fractions (carbohydrates, lipids, protein, and lignin) of the enriched manure processes. Finally, a preliminary techno-economic analysis of the proposed process is presented.

2. Materials and Methods

2.1. Feedstock and Inoculum

The swine manure used in the experiments was collected from Hashøj biogas plant (Sjæland, Denmark) and was stored in closed containers at −20 °C until used. The manure collected originated from different local pig farms, thus containing manure from swine production at different stages, as classified in Cestonaro do Amaral et al. [16]. The swine manure fibers were separated in farm from raw swine manure by means of a mobile decanter centrifuge and transported to Limfjordens Bioenergi (Mors, Denmark), where they were collected. The manure fibers were stored in sealed freezer bags and kept at −20 °C until used for the experiments. The inoculum used for initiating the AD experiments originated from a 3 L lab-scale continuous stirred-tank reactor (CSTR) type digester operating on manure. The main characteristics of the inoculum were 3.81% total solids (*TS*), 2.32% volatile solids (*VS*), 2.39 g $NH_4^+ - N$/L, 2.26 g soluble chemical oxygen demand (*COD*)/L, and *pH* 8.0.

2.2. AAS Pretreatment

The AAS pretreatment of the swine manure fibers was performed at ambient temperature (20 °C) and under optimal conditions (7% NH_3 w/w, 4 days, 0.16 kg fibers per liter of ammonia solution) in sealed flasks according to previous results and as described in Lymperatou et al. [14]. After the end of the pretreatment, a vacuum evaporation procedure was followed at 130 mbar while the water bath temperature was increased progressively to 80 °C for reducing the concentration of $NH_4^+ - N$ of the pretreated fibers to less than 0.5 g/L. Prior to the evaporation, tap water of equal volume to reagent was added to the pretreated fibers.

2.3. Experimental Set Up of Continuous AD

Three CSTR-type digesters (3 L active volume) were used in the present study; all operated under mesophilic conditions (37 °C). The first digester was fed only with swine manure serving as a reference process (reference digester) for obtaining background data on the CH_4 productivity and yield of a process with similar characteristics fed only with manure. The inoculum for the digester′s start-up originated from the effluent of a lab-scale digester running on swine manure for 3 years under mesophilic conditions. After 120 days of operation of the reference digester, the second (NP digester) and third digester (AAS digester) were inoculated simultaneously with a mixture of liquid from the reference digester and accumulated effluent also from the reference digester. The NP and AAS digesters were fed with a mixture of swine manure and manure fibers with a swine manure to fibers ratio of 2:1 (TS basis), where the fibers were non-pretreated (NP) and optimally AAS-treated respectively. The feed for the NP digester was diluted with tap water in order to achieve a similar *TS* content and organic loading rate (*OLR*) to the AAS digester. The experiments with the NP and AAS digesters lasted 125 days.

All digesters were fed once per day by means of peristaltic pumps after rigorous mixing of the feed for 15 min. Feed mixtures were prepared twice per week and the feed tanks were kept at 4 °C to reduce microbial degradation prior to digestion. Stirring of the digesters was intermittent and took place every 3 h for 10 min. The heating of the digesters was achieved by water jackets with recirculating water from a thermostatic water bath.

Biogas production was measured with Ritter MilliGascounters (Ritter, Germany). The CH_4 content of the produced biogas, volatile fatty acids (*VFA*s), soluble *COD*, $NH_4^+ - N$ content, and *pH* were monitored weekly in all digesters. Samples for *TS* and *VS* determination were also collected weekly from the influent and effluent streams as well as from inside the digesters. The total suspended solids (*TSS*) concentration inside the digesters and in the effluents was determined every two weeks. The solids retention time (*SRT*) was higher than the hydraulic retention time (*HRT*) in all digesters due to a part of the effluent pipe that was vertical, permitting the re-settling of the solids when the digesters were not fed [17]. The *SRT* was estimated based on the *TSS* measurements of samples taken from inside the digesters and from the effluents, as:

$$SRT = \frac{TSS_{reactor} \cdot V_{reactor}}{Q_{out} \cdot TSS_{effluent}} \tag{1}$$

where: *SRT* is the solids retention time in days; $TSS_{reactor}$ and $TSS_{effluent}$ are the concentrations of *TSS* in g/L inside the reactor and in the effluent respectively; $V_{reactor}$ is the active volume of the reactor in L; and Q_{out} is the flow rate of the effluent in L/d.

2.4. Analytical Methods and Compositional Analysis

TS, *VS*, *TSS*, volatile suspended solids (*VSS*), and fixed solids (ash content) were determined according to Standards methods [18]. Determination of soluble compounds was performed after centrifugation of samples at 10,000 rpm for 10 min and subsequent filtration of the supernatant through 0.45 µm. $NH_4{}^+ - N$, soluble K, and soluble *COD* concentrations were quantified by Hach Lange kits LCK 305, LCK 328, and LCK 514, respectively. The phosphorus determination was performed by following the ascorbic acid method as described in Edwards et al. [19]. Free NH_3 content was calculated as [3]:

$$FAN = TAN \cdot \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{273.15+T}\right)}}\right)^{-1} \tag{2}$$

where: *FAN* is the free ammonia concentration (NH_3) in the digester in g/L; *TAN* is the total ammonia concentration ($NH_4^+ + NH_3$) in the digester in g/L; and *T* is the temperature of the digester in °C.

Determination of CH_4 content in biogas was carried out by gas chromatography (GC82-22, Mikrolab Aarhus, Denmark). The GC was equipped with a Porapak Q packed column (6 ft and I.D. 3 mm), coupled with a thermal conductivity detector and N_2 was used as a carrier gas. All gas volumes reported correspond to STP conditions (0 °C, 1 atm).

Compositional analyses of manure and digester effluents were performed on samples taken during the period of 70–80 days of operation of the digesters. The procedure followed for quantification of structural carbohydrates, acid-insoluble (Klason) lignin, water and ethanol extractives, and soluble sugars of the samples is described in Lymperatou et al. [14]. The composition of the influents of the NP and AAS digesters was calculated based on the composition of the manure fibers and the composition of raw manure used and taking into account the *TS* ratio of manure and manure fibers. Values reported for cellulose and hemicellulose correspond to the sum of glucose and sum of xylose and arabinose respectively. Samples for *VFA*s analysis were acidified with H_2SO_4 (10% w/w), centrifuged at 10,000 rpm for 10 min and filtered through 0.20 µm. Determination of sugars and *VFA*s was performed by high performance liquid chromatography (HPLC) equipped with a refractive index and an Aminex HPX-87H column (BioRad) at 63 °C as described previously [20]. A solution of 12 mM H_2SO_4 was used as an eluent at a flow rate of 0.6 mL/min. Total N content was determined through elemental analysis (EA3000, EuroVector, Italy) with acetanilide used as a standard. The proteins content was determined by subtracting the *TAN* content from the total N content and multiplying by the factor 6.25 [21]. The lipids were determined as the mass of extractives after 24 h of extraction with ethanol 96% *v*/*v* using a Soxhlet apparatus (EV6 ALL/16 No. 10-0012, Gerhardt, Germany).

2.5. Theoretical Calculations and Assumptions

The reduction efficiencies of the major biomass components (cellulose, hemicellulose, proteins, lipids, and lignin) were calculated as:

$$\%\ reduction = \frac{x_{i,\ in} - x_{i,\ out}}{x_{in}} \cdot 100 \tag{3}$$

where: $x_{i,\ in}$ the concentration of each component i in g/kg in the influent; and $x_{i,\ out}$ the concentration of each component i in g/kg in the effluent.

The theoretical CH_4 yield was calculated based on the composition and according to Buswell's formula [22]:

$$\mathrm{C_nH_aO_b} + \left(\mathrm{n} - \frac{\mathrm{a}}{4} - \frac{\mathrm{b}}{2}\right)\mathrm{H_2O} \rightarrow \left(\frac{\mathrm{n}}{2} - \frac{\mathrm{a}}{8} + \frac{\mathrm{b}}{4}\right)\mathrm{CO_2} + \left(\frac{\mathrm{n}}{2} + \frac{\mathrm{a}}{8} - \frac{\mathrm{b}}{4}\right)\mathrm{CH_4} \tag{4}$$

The molecular formulas for each component were assumed to be $C_6H_{10}O_5$ for cellulose, $C_5H_{10}O_5$ for hemicellulose, $C_5H_7O_2N_1$ for proteins, $C_{57}H_{104}O_6$ for lipids, and $C_2H_4O_2$ for *VFA*s, as suggested by Møller et al. [23].

The hydrolysis constants k_h of NP and AAS manure fibers were calculated by fitting a 1st order model to the CH_4 production of biochemical methane potential (BMP) tests, as described in Lymperatou et al. [11]. Based on the k_h calculated, the following equation was used for predicting the CH_4 yield of manure fibers in continuous AD [24]:

$$B = B_0 \cdot \left(1 - \frac{1}{1 + k_h \cdot t}\right) \tag{5}$$

where: B, CH_4 yield of substrate after t days of digestion in mL/g TS; B_0, ultimate CH_4 yield in mL/g TS; k_h, the hydrolysis rate in d^{-1}; and t, the *SRT* in d.

2.6. Techno-Economic Analysis

The proposed process in this study involves the integration of the AAS pretreatment and NH_3 recovery to a manure AD process. In order to evaluate the increase of energy recovery compared to manure mono-digestion and to the AD of manure enriched with NP manure fibers, the process was simulated with Aspen HYSYS ® (Version 9, ASPEN Technology Inc., Bedford, MA, USA). Process simulations were carried out targeting the conditions applied at laboratory scale and assumptions were made based on experimental data and on reference values reported in literature when necessary. The increase of revenue was calculated based on the comparison of 3 scenarios; the first scenario corresponding to the mono-digestion of 10 kg liquid swine manure, the second scenario representing the AD of 10 kg of liquid swine manure and 3 kg of NP manure fibers, and the third scenario corresponding to the AD of 10 kg liquid swine manure and 3 kg of manure fibers pretreated by AAS including an NH_3 recovery process. The above quantities have been chosen as a basis for presenting the increase of revenue at the given ratio. Upscaling of the process could be applied by simply keeping the ratio of manure to fibers constant.

3. Results and Discussion

3.1. Process Characteristics, Productivity, and Stability

The main composition of the swine manure and manure fibers used in this study is presented in Table 1. In general, the substitution of 1/3 of the dry matter of the influent with manure fibers resulted in a slight increase of organic matter in both digesters as compared to the reference digester. The swine manure presented an average content of organic matter, *VS* (68% TS), as compared to values found in literature (60–77% TS) [25–29]. The organic fraction of the NP manure fibers was of the same magnitude as in swine manure; however they were significantly richer in carbohydrates (cellulose and hemicellulose). Even though the fibers did not originate from the same batch of manure, this was expected as during solid-liquid separation, the sugars that are bound to the biomass remain in the solids resulting thus in a greater fraction of the dry matter. The composition of the pretreated fibers showed some differences in comparison to the NP fibers especially in regards to the hemicellulose and protein content, affecting the overall influent composition in the NP and AAS digesters. During optimal conditions of AAS at ambient temperature, partial solubilization of hemicellulose takes place while the cellulose content remains the same [11,14]. The solubilized hemicellulose was partly converted to oligosaccharides and monomeric sugars and partly to degradation products not detected by the methods applied. An increase of the protein content was also observed in the AAS fibers, which could be attributed to the formation of N-complexes during the pretreatment, as a small fraction of the reagent N is bound to the biomass [14]. AAS also resulted in a reduced lipid content of manure fibers, implying that saponification might have occurred. Overall, aside from these differences, the organic fraction of the manure fibers was similar in both NP and AAS fibers, corresponding to ca. 70% TS, resulting in similar *OLR*s in the two digesters (Table 2).

Table 1. Composition of manure and non-pretreated (NP) and aqueous ammonia soaking (AAS)-treated fibers used in this study.

Component	Swine Manure	Non-Pretreated Manure Fibers [2]	AAS-Treated Manure Fibers [2]
TS (% wet mass)	2.2	3.2 [1]	3.1
VS (% wet mass)	1.5	2.2	2.2
Cellulose (% TS)	12.3	30.4	31.2
Hemicellulose (% TS)	9.2	21.7	16.1
Proteins (% TS)	22.9	15.1	19.9
Lipids (% TS)	7.7	7.8	5.9
Lignin (% TS)	15.8	16.6	16.7
TAN (% TS)	1.09	0.37	0.99

[1] After dilution with tap water [2] adapted from Lymperatou et al. [14].

Table 2. Process characteristics of digesters during the period of 65–95 days of operation.

Characteristic	NP Digester	AAS Digester	Reference Digester
Feed ratio g TS manure: g TS fibers	2:1	2:1	1:0
C/N_{org} of influent	11.1	10.2	9.4
Organic Loading Rate (g VS/L/d)	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.1
Hydraulic Retention Time (d)	18.2 ± 1.1	17.9 ± 1.3	17.6 ± 0.1
Solid Retention Time (d)	26.7 ± 1.1	25.9 ± 1.9	20.5 ± 0.3
VFA concentration [1] (g/L)	0.19 ± 0.07	0.26 ± 0.07	0.22 ± 0.01
pH[1]	8.1 ± 0.0	8.1 ± 0.1	8.2 ± 0.3
Soluble *COD* [1] (g/L)	2.24 ± 0.07	2.34 ± 0.23	3.08 ± 1.34
TAN concentration [1] (g/L)	1.82 ± 0.26	2.04 ± 0.01	2.73 ± 0.05
FAN concentration [1] (g/L)	0.25 ± 0.03	0.29 ± 0.01	0.46 ± 0.01
TS[1] (g/L) inside the digester	26.63 ± 0.95	22.17 ± 1.53	26.61 ± 1.00
VS[1] (g/L) inside the digester	16.40 ± 0.69	13.24 ± 1.01	15.29 ± 0.83
TSS[1] (g/L) inside the digester	20.35 ± 0.07	17.05 ± 0.07	16.70 ± 0.64
Biogas productivity (L/L/d)	0.41 ± 0.08	0.48± 0.06	0.43 ± 0.06
Methane productivity (L/L/d)	0.25 ± 0.04	0.30 ± 0.04	0.28 ± 0.04
CH_4 (% biogas)	61.8 ± 0.9	64.1 ± 1.1	66.0 ± 3.0
Methane yield (mL/g TS_{fed})	156 ± 37	215 ± 40	204 ± 34
Methane yield (mL/ g VS_{fed})	222 ± 54	314 ± 61	330 ± 61

[1] Data correspond to average values from samples taken from inside the digesters accompanied by the standard deviation.

Swine manure often contains high *TAN* concentrations, which in combination with the mineralization of organic N during AD, increases the risk of partial inhibition of the methanogenesis step [3]. Therefore, mono-digestion of this feedstock is often discouraged [30]. The *TAN* concentration in the reference digester, which was fed only with swine manure, was 2.73 g/L. The addition of fibers, both in the NP and AAS digester, led to a reduction of the *TAN* concentration (Table 2). This was expected, as aside from the lower *TAN* concentration of the fibers (Table 1), the mixture-based digesters had a lower organic N content as indicated by their protein content (Table 1) and by the C/N_{org} ratios (11.08 and 10.24 for the NP and AAS digester, respectively, in comparison to 9.44 for the reference digester), (Table 2). Generally, the threshold value reported in literature at which NH_3 inhibition begins varies significantly and is dependent on the *pH*, the temperature, the *TAN* concentration, the presence of other ions and the acclimation of the inoculum [31,32]. According to a recent review [33], a *TAN* concentration above 1.7–1.8 g/L is inhibitory under mesophilic conditions without acclimation of the inoculum. The NP digester was the only digester with such low concentration. However, as the initial inoculum originated from a digester operated for 3 years on swine manure with *TAN* concentrations similar to the reference digester′s concentration used, the inoculum could be considered well acclimated [34]. Research has shown that both NH_4^+ and NH_3 may produce inhibition to methanogenic cultures. The inhibition mechanism for each type differs; high concentration of NH_4^+ is considered to produce inhibition of enzymes producing CH_4, while the inhibition mechanism of NH_3 (*FAN*) is based on its diffusion through the cell membrane producing proton imbalance and deficiency of K^+ [35]. Among the two forms, *FAN* is widely considered to be a stronger and more direct inhibitor [32]. However, there is no universal threshold for *FAN* inhibition reported in literature due to the complexity of the involved factors in different systems [32]. Inhibition due to *FAN* is usually reflected in accumulation of *VFA*s as the main group affected by *FAN* is the methanogenic archaea. However, the concentration of *VFA*s was very low during the entire period of experiments in all processes in this study (Table 2), which further supports the acclimation of the inoculum.

The AAS digester generally presented an improved biogas production compared to the rest of the digesters. The average biogas productivity of the AAS digester was 0.48 L/L/d, corresponding to a 17% increase as compared to the productivity of the NP digester (0.41 L/L/d), and a 12% increase in comparison to the reference digester (0.43 L/L/d). The inhomogeneity of the feed had as a result a variability in the daily observed production and yield data; however, as shown in Figure 1, the AAS digester performed consistently better than the NP digester. Interestingly, the NP digester presented lower biogas productivity than the reference digester, indicating that reducing the *TAN* (and *FAN*) concentration was not sufficient for improving the conversion efficiency of the process. This implies that the degradability of manure was a more important factor limiting the biogas production than the *TAN* (and *FAN*) concentration. The inoculum used for the digesters startup originated from a long-term AD process of liquid manure and thus it was better acclimated for the reference digester process than for the mixture-based digesters. However, given that liquid manure already contains manure fibers (in a lower percentage), a difference in biogas production due to the lack of inoculum acclimation would not be expected to be significant. The CH_4 productivity of the digesters presented similar trends to the biogas productivity, with the AAS digester performing better than the rest of the digesters. Nevertheless, the addition of fibers (both NP and AAS-treated) resulted in a reduction of the content of CH_4 in biogas (Table 2). This was probably a result of the higher fraction of carbohydrates in the mixture-based digesters, that stoichiometrically produce a lower CH_4/CO_2 ratio in comparison to lipids and proteins in which manure was richer (Table 1). Still, the highest CH_4 yield per g TS_{fed} was observed in the AAS digester.

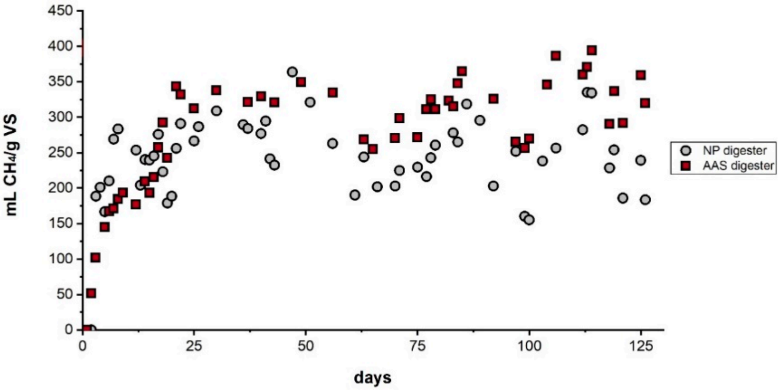

Figure 1. Methane yield of digesters NP (fed with manure and NP fibers) and AAS (fed with manure and AAS-treated fibers) during the entire period of experiments.

The evolution of the CH_4 yield of the mixture-based digesters during the entire course of experiments is shown in Figure 1. The theoretical maximum CH_4 yields of the mixture-based digesters were calculated to be 377 mL/g TS_{fed} (538 mL/g VS_{fed}) and 365 mL/g TS_{fed} (533 mL/g VS_{fed}) for the NP and AAS digester, respectively, while the yields obtained experimentally were 156 mL CH_4/g TS_{fed} for the NP digester and 215 mL CH_4/g TS_{fed} for the AAS digester. These correspond to 41% and 59% of the theoretical yields, respectively, and to a 38% increase of the CH_4 yield from the AAS digester compared to the NP digester. Assuming that the hydrolysis is the limiting step in the AD of manure fibers, a 1st order model was fitted to the CH_4 production of NP and AAS manure fibers of previously run batch experiments [14] (data presented in Supplementary material). This permitted calculating the hydrolysis constants k_h (0.1187 d^{-1} for NP fibers and 0.1329 d^{-1} for the AAS fibers), based on which, and by taking into consideration the *SRT* of each digester, the expected yields of NP and AAS fibers in the continuous experiments (without manure) were calculated to correspond to 55 mL/g TS_{fed} and 161 mL/g TS_{fed}, respectively by using Equation (5). This corresponds to an increase of the CH_4 yield of the fibers of 193%. Given the feed ratio of the mixture-based digesters (2 g TS manure/ 1 g TS fibers) and assuming that the CH_4 yield of manure corresponded to the same as in the reference digester (204 mL/g TS_{fed}), the expected CH_4 yields of the mixture-based processes (manure and fibers) were calculated to be 151 mL/g TS_{fed} and 189 mL/g TS_{fed} for the NP and AAS digester, respectively. The theoretical predictions were similar to the experimental measurements for the NP digester, while the AAS digester exhibited a slightly higher efficiency experimentally (12%) in regards to the CH_4 yield. Therefore, the 1st order model was safely used for calculating the yields during the process simulation in HYSYS (Section 3.3).

In the present study, the processes were operated at low *OLR*s because of restrictions of the lab-scale pumping system used, making it not possible to increase the share of fibers in the feed due to clogging of the feeding tubing. While it could be expected that certain instability would be produced by increasing the *OLR* and the share of fibers [29], this would be largely associated to the availability of the added C and N. In this sense, the addition of fibers with an increased N content could be balanced if the C of the fibers is available for microbial uptake. Thus, if no further pretreatment of fibers with high N concentration is followed, their use for enriching liquid manure might result to be prohibitive. In a previous study, where raw manure fibers were added to pig manure, a significant increase in the *TAN* concentration was observed, producing inhibition of the process when increasing the substitution of manure with fibers up to 60% [28]. This difference was mainly attributed to the higher total N concentrations of manure fibers in comparison to raw manure [28]. The efficiency of separating the organic N content of liquid manure depends on the technology used and, generally, it is increased with an increased efficiency of solids separation [36,37]. On the other hand, the *TAN* concentration remains in the liquid fraction regardless of the separator used [36]. The separation and AAS pretreatment of fibers for the enriching of raw manure can provide certain flexibility on the final total N content of the influent, given that an NH_3 removal step is necessarily applied after AAS and before AD. The source of manure fibers and the efficiency of solids separation could determine whether the organic N in the fibers is lower than in manure, while the NH_3 removal step following an AAS pretreatment would allow for a better control of the initial *TAN* concentration of the feed.

3.2. Reduction Efficiency of Major Organic Components and Digestate Quality

The composition of the effluents of the NP and AAS digesters was analyzed in order to better estimate the effects produced by the optimized AAS pretreatment. The efficiency of VS reduction of the NP and AAS digesters along with the reduction efficiencies of the major organic components are shown in Table 3. The AAS digester presented a higher efficiency on reducing the organic matter of the feed (50.7% reduction of VS) than the NP digester (45.8%). It is important to mention here that the VS increase due to the growth of microbial biomass has not been taken into account for the calculations. However, as the aim of this study was to compare the reduction efficiencies of the different organic components in the two digesters (NP and AAS), this was considered not to affect the evaluation significantly. Among the two processes, the AAS digester presented higher reduction efficiencies in all major organic components of the feed. The highest difference was observed in the carbohydrate fraction where the digestion of manure enriched with pretreated fibers resulted in a 60.3% and 65.3% reduction of the cellulose and hemicellulose fractions, respectively, in comparison to 42.6% and 54.1% from the digestion of manure with NP fibers. Therefore, a 42% and 21% increase of reduction efficiency for cellulose and hemicellulose was achieved, respectively, due to the AAS pretreatment. This was expected as AAS affects mostly the lignocellulosic fraction of the biomass, by increasing the efficiency of polysaccharide hydrolysis. As reported in earlier studies, the main mechanism of AAS on swine manure fibers appears to be a swelling effect [38] together with a significant solubilization of the hemicellulose fraction [14] resulting thus in both the cellulose and hemicellulose fractions being more accessible for microbial degradation.

Table 3. Concentration of major organic components of influents and effluents of digesters and reduction efficiency of anaerobic digestion (AD) (data correspond to samples taken during 70–80 days digestion).

	NP Digester			**AAS Digester**		
Component	**Influent (g/kg)**	**Effluent (g/kg)**	**% Reduction**	**Influent (g/kg)**	**Effluent (g/kg)**	**% Reduction**
VS	21.87 ± 0.53	11.9 ± 1.93	45.8	20.32 ± 1.96	10.02 ± 2.16	50.7
Cellulose	4.76 ± 0.00 [1]	2.74 ± 0.02	42.6	4.53 ± 0.11 [1]	1.80 ± 0.11	60.3
Hemicellulose	3.83 ± 0.01 [1]	1.76 ± 0.02	54.1	2.81 ± 0.01 [1]	0.98 ± 0.06	65.3
Proteins	6.21 ± 0.79 [1]	3.70 ± 0.03	40.5	6.25 ± 1.20 [1]	3.27 ± 0.03	47.7
Lipids	2.37 ± 0.18 [1]	1.52 ± 0.13	35.6	2.01 ± 0.01 [1]	1. 11 ± 0.19	44.6
Lignin	4.92 ± 0.36 [1]	3.03 ± 0.00	38.5	4.61 ± 0.35 [1]	2.39 ± 0.10	48.2
VFAs	6.52 ± 0.54	0.19 ± 0.07	97.1	6.20 ± 0.42	0.26 ± 0.07	95.8

[1] Estimated through mass balance.

Aside from the improved carbohydrate removal, AAS appears to have facilitated the reduction of lipids and proteins as well. A slight solubilization of organic N (associated to proteins) and decrease of ethanol extractives (associated to lipids) were observed after AAS [14], probably facilitating their further degradation. The reduction of lignin was also significantly affected by the AAS treatment, as it reached 48.2% in the AAS digester in comparison to 38.5% in the NP digester. It is known that lignin is not significantly removed from manure fibers after optimal AAS at ambient temperature [14], thus this could be a result of the swelling of the fibers that facilitated microbial access during AD resulting in degradation products. Generally, lignin is considered to be recalcitrant to bioconversion and negatively correlated to CH_4 production [39,40]. Nevertheless, upon degradation, certain byproducts have been reported to be converted into CH_4 under anaerobic conditions [41].

Energy production is undoubtedly a very important asset of AD that has led to an increased focus on this technology lately. Nevertheless, another aspect of AD that deserves attention is the digestate (digester effluent) valorization. During AD the organic matter content of the substrate is reduced to a certain degree, resulting in a digestate that contains non-degraded fractions together with valuable nutrients that can be useful for further applications. For instance, organic N is converted to $NH_4^+ - N$ increasing thus the availability of this essential nutrient for bacteria, algae, and plants. Similarly, the solid fraction presents the recalcitrant fractions that still contain C that can be converted to energy through thermochemical conversion, e.g., pyrolysis, combustion, gasification, etc. or serve as a slow-release C source in agricultural soils. While research on alternative applications of digestate is expected to advance in the coming years [42], currently it is mostly used as a soil amendment and crop fertilizer.

Some basic characteristics of the digestates (shown in Table 4) can indicate their stability as well as whether further processing should take place prior to their integration in agricultural systems. The main elements of organic matter are C and N, and consequently a mature organic amendment should have these elements as stabilized as possible. Different criteria have been proposed for qualifying organic amendments such as compost and digestates. The ratio of cellulose/lignin has been proposed as an indicator of humic acids formation in soil and a ratio lower than 0.5 has been proposed to characterize a mature amendment [43,44]. Among the digestates of the enriched manure, the effluent of the AAS digester presented a lower cellulose/lignin ratio than the effluent of the NP digester (Table 4) as expected due to more extended degradation of carbohydrates. The values of both digestates stand above the threshold mentioned, probably due to the degradation of lignin resulting in a simultaneous reduction of both carbohydrates and lignin. This indicator, however, does not take into account the total amount of remaining matter that can still be oxidized. As indicated by the *COD/TS* ratio of the digestates (0.1 and 0.2 for the NP and AAS digestates, respectively) and based on the criterion of stability of 0.7 g COD/g TS [43], the total content of the remaining fractions that could undergo oxidation is satisfactory. From a *C/N* point of view, both digestates corresponded to values below 15, thus no alteration of microbial equilibrium of well-balanced soils should occur according to Bernal et al. [45].

Table 4. Quality parameters of NP and AAS digester effluents for soil application.

Quality Parameter	NP Effluent	AAS Effluent
TS (% wet mass)	2.0 ± 0.3	1.8 ± 0.3
Total *COD* (g/g TS)	0.1 ± 0.0	0.2 ± 0.0
VS (% TS)	58.5 ± 0.7	56.8 ± 3.6
pH	7.9 ± 0.1	7.9 ± 0.1
C/N	8.9 ± 0.0	8.4 ± 0.1
Cellulose/Lignin	0.90 ± 0.0	0.75 ± 0.0
$NH_4^+ - N$ (% Total N)	73.8 ± 3.2	77.3 ± 1.9
$NH_4^+ - N$ (% TS)	7.6 ± 1.2	11.1 ± 1.9
Soluble *COD* (% Total COD)	72.7	63.2
Soluble *C/N* ratio	2.4 ± 0.2	2.7 ± 0.3
Soluble total N (g/L)	2.6 ± 0.1	2.9 ± 0.0
Soluble $NH_4^+ - N$ (g/L)	1.7 ± 0.0	1.8 ± 0.1
Soluble $PO_4^{3-} - P$ (g/L)	0.1 ± 0.0	0.1 ± 0.0
Soluble $K^+ - K$ (g/L)	5.1 ± 0.2	5.2 ± 0.4

The values of the $NH_4^+ - N$ fraction of the total N of the digestates indicate that probably their use as fertilizers would be more appropriate than as soil amendments [44]. This characteristic of the digestates is both due to the degradation of proteins during AD and the high $NH_4^+ - N$ concentration of the original feedstock, swine manure. Regarding the fertilizing value of the digestates, the soluble N, P, and K nutrients are considered to be readily available to plants [43]. As expected due to the origin of the feedstock, high values of N and K and a low concentration of P were found in the soluble fraction (Table 4). The N and K concentrations are similar to values reported elsewhere on liquid manure-based digestates [46]. The soluble P is relatively low, which could be explained by the fact that usually the majority of P remains in the solid fraction of digested manure [47], however it is also similar to values reported elsewhere [44]. Finally, the pH values of the digestates are close to neutral, being thus compatible with most of plants.

In conclusion, the digestates of the processes presented in this study can be valuable for agricultural valorization under certain conditions. The differences among the characteristics of the digestates were very small (Table 4), indicating that AAS does not affect the quality of the digestate negatively. The high fraction of $NH_4^+ - N$ indicates that the digestates should be treated as fertilizers rather than as soil amendments. Nevertheless, it is common to separate anaerobic digestates into a liquid and solid fraction in order to facilitate and make their management independent. In such cases, the solid fraction can be used as an amendment while the liquid fraction as a fertilizer. Even under these conditions, the high $NH_4^+ - N$ concentration might cause phytotoxicity according to Teglia et al. [43]. This could be avoided by optimizing the NH_3 removal and recovery of AAS-treated fibers prior to AD, reducing thus the $NH_4^+ - N$ concentration of the digestate and improving the performance of the AD process. Additionally, the surplus NH_3 of the effluent could be recovered and used for the chemical needs of the pretreatment [13], as also discussed in Section 3.3.

3.3. Preliminary Techno-Economic Analysis of AD Coupled to AAS and NH_3 Recovery

Based on the experimental results obtained so far, the AD process coupled to the AAS pretreatment of manure fibers and an NH_3 recovery process was simulated in HYSYS. The purpose of this analysis was to estimate the increase of biogas production due to AAS along with some key output values by setting target values for some important characteristics of the process (Table 5), ultimately for presenting the potential and some important limitations of the proposed process.

Table 5. Input/output/target conditions for modeling in HYSYS.

Process Characteristics	Units	Values	Input/Output/Target
HRT	(days)	20	Input
Fibers/wet mass input	(kg)	3	Input
TS in received manure fibers	(% mass)	30	Input
NH_3 in received manure fibers	(% mass)	0.2	Input
Fresh manure mass input	(kg)	10	Input
TS in fresh manure	(% mass)	6	Input
NH_3 in fresh manure	(% mass)	0.7 (0.4) [1]	Input
TS in mixture for NH_3 extraction	(% mass)	<12	Input
Vacuum distillation pressure	(bar abs)	0.13	Input
Assumed TS conversion biogas reactor	(% mass)	25	Input
Steam input (250 °C)	(kg)	1.0	Input
NH_3 in AAS treatment	(% mass)	ca. 6	Target
TS in AAS treatment	(% mass)	ca. 4	Target
NH_3 in mixture after extraction	(% mass)	<0.5	Target
Internal heat exchange	(MJ)	3.0	Output
Energy for compressor	(kJ)	485	Output
NH_3 extraction temperature	(°C)	47	Output
AAS pretreatment temperature	(°C)	14	Output
Calculated total TS conversion rate	(% mass)	36	Output
Biogas output energy with AAS	(MJ)	10.70	Output

[1] NH_3 content in swine manure is increased from 0.4% to 0.7% to account for NH_3 formation after AD.

Figure 2 shows the process flow diagram based on the HYSYS simulation. The manure fibers are delivered to the biogas plant where they undergo pretreatment targeting the optimal AAS conditions [14]. After AAS, the pretreated fibers are subjected to a mechanical separation step in order to recover the majority of NH_3 in the liquid fraction and reuse it for subsequent pretreatments. This was a necessity as the energy demand for extracting NH_3 from the whole pretreated batch would be excessive due to heating requirements of the high liquid volume. The pretreated fibers still presenting a high NH_3 concentration for AD after separation are sent to an NH_3 extraction step, after which the concentration of NH_3 is targeted to be less than 0.5%. The NH_3 extraction takes place at 130 mbar and is facilitated by the injection of steam at 250 °C. In order to ensure the NH_3 needed for the pretreatment implementation and for adjusting the solids concentration of the NH_3 extraction step of the fibers, part of the liquid fraction of the effluent of the digester (which presents an increased NH_3 concentration) is introduced in the extraction step. The extracted NH_3 is condensed and sent back to the AAS pretreatment tank for covering the chemical needs of the pretreatment. The fibers after NH_3 extraction are inserted to an anaerobic digester along with swine manure sent from the farms, which is operated at continuous mode. The dry matter content of the digester is thus increased to ca. 9% compared to 6% if solely manure would be digested. After AD, the digestate is sent back to the farmers for crop fertilization. During NH_3 extraction, CO_2 is also extracted from the system, which is then sent to the effluent stream for avoiding CO_2 build up in the extraction process. A very small part of the NH_3 recovered (0.1%) from the extraction step is used for the catalytic reduction of NO_x emissions from gas engines as described previously [13], providing thus a more environmental friendly electricity production.

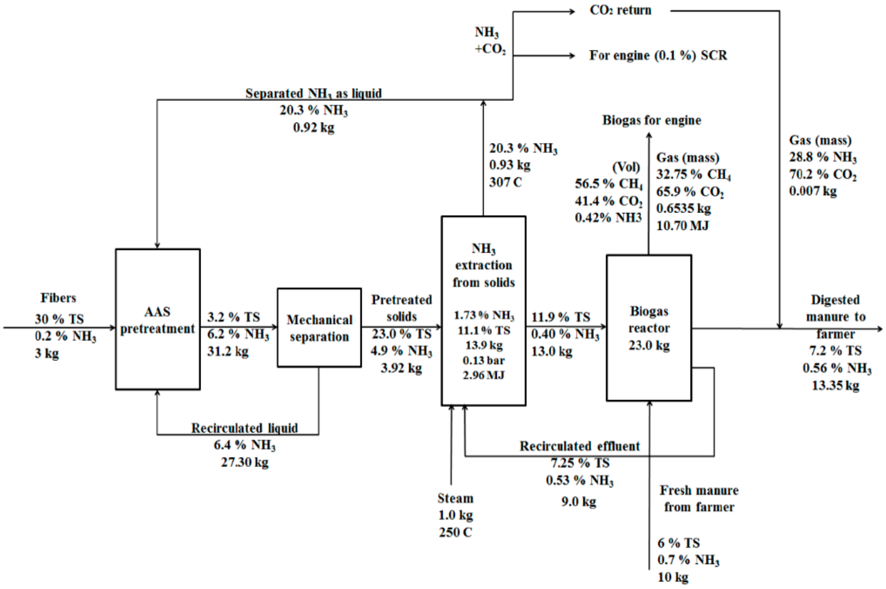

Figure 2. AD process of swine manure coupled with AAS pretreatment of manure fibers and NH_3 recovery. All mass and energy units are expressed per hour.

To facilitate the simulation process, some necessary assumptions were made. Based on the simulation presented in Figure 2, the temperature of the pretreatment would correspond to 14 °C. According to preliminary experiments of AAS of manure fibers at 4 °C and 25 °C, there was no significant difference in the resulting CH_4 yield (data shown in Supplementary material), thus this setting was considered to be acceptable. The efficiency of AAS with recirculated NH_3 is expected to be similar compared to the efficiency when using NH_3 diluted with tap water. To this end, preliminary experiments showed that when centrifuged effluent was added to the NH_3 evaporation process instead of tap water (see Section 2.2), no significant difference was observed in the CH_4 yield as evidenced from BMP tests (data shown in Supplementary material). However, other assumptions as of the stability of the process at increased *OLR* and the effect of the removal of part of the soluble organic matter (that is recirculated to the AAS pretreatment after solid-liquid separation) prior to AD still remain to be tested at a larger scale. The solubilized fraction of AAS fibers has been measured to correspond to 14.62% *COD* of the total COD [14]. However, as degradation products are formed in the soluble fraction, their fate during AD is not known and thus their share in the final CH_4 yield cannot be directly calculated. Finally, the energy calculations were based mainly on the heat from condensation and steam requirements while the heating losses have not been taken into account.

The ultimate goal of the simulation was to have an estimation of the energy gain from the integration of AAS and NH_3 recovery to the process. The simulation showed that the biogas output from treating 10 kg manure with 3 kg manure fibers pretreated with AAS (scenario 3) would correspond to 10.70 MJ. The steam preparation and the compressor would require 2958 kJ and 485 kJ, respectively, while 2687 kJ can be recovered from the NH_3 condensation unit through heat transfer. Thus, a 756 kJ input is required for the implementation of the process, which corresponds to 7.0% of the total energy output (10.7 MJ) from the AD of manure and AAS-treated fibers. By using the same input data for the CH_4 yields of manure and NP fibers and the same *HRT* as for the simulation

(20 days), it was calculated that the energy produced from the AD of manure and NP manure fibers (scenario 2) would correspond to 7.04 MJ, while that of solely manure (scenario 1) would correspond to 5.17 MJ (calculations are presented in Supplementary material). Given that the price of biogas in Denmark corresponds to 0.1765 DKK/MJ (including subsidies) [48,49], a 72% increase of revenue would be achieved by integrating AAS and NH_3 recovery compared to the AD of manure and NP fibers, and a 135% increase compared to the revenue from digesting only manure (Table 6). In this simulation, a manure to fibers TS ratio of 2:3 was applied, resulting in approximately 9% TS in the anaerobic digester. However, CSTR-type digesters may permit a solid loading of up to 12% without the need to replace the pumping and stirring infrastructure [50,51]. Thus, a higher share of fibers could be permitted in existing full-scale liquid AD installations. As discussed in Section 3.1, the quality and composition of manure and manure fibers are of great importance on the success of the proposed process. Nevertheless, given the above-mentioned considerations, and the flexibility the NH_3 recovery process offers to the final *C/N* ratio of the AD process, a higher share of AAS-treated manure fibers is expected to result in a significantly higher improvement of biogas production. While further research should investigate different ratios of manure fibers to manure, as well as to address the potential deviations derived from the assumptions made, this preliminary estimation indicates that the proposed process can be viable and result in an impressive increase of revenue.

Table 6. Energy output and revenue for different scenarios.

Scenario	MJ Produced	DKK per kg Digested	% Increase of Revenue due to AAS
1-Swine manure (10 kg)	5.17	0.091	+135
2-Swine manure (10 kg) + NP fibers (3 kg)	7.04	0.096	+72
3-Swine manure (10 kg) + AAS manure fibers (3 kg)	10.70	0.145	-

4. Conclusions

This study investigated the potential of improving continuous manure-based AD with the addition of optimally AAS-treated manure fibers. The results from lab-scale experiments showed that a 38% increase of the CH_4 productivity is possible at a 2 g TS manure per 1 g TS manure fibers feed ratio, as compared to the AD of manure and manure fibers without AAS. Moreover, a significantly improved reduction of all the major organic components in the digestate was observed due to AAS, improving thus the digestate quality for land application. By comparison of the processes to a digester fed solely with liquid manure, it was shown that the biodegradability of the fiber fraction of manure was a more limiting factor impeding the biogas production than the initial high NH_3 concentration. The coupled AAS-AD process was satisfactorily simulated in HYSYS, and a 72% increase of revenue was calculated, compared to the simple addition of untreated manure fibers to manure AD at a TS ratio of 3:2 of initial feedstock composition, respectively. The simulation indicated that future research should focus on the effect of solid separation of pretreated manure fibers prior to NH_3 recovery, with regard to both the resulting CH_4 yield after liquid separation, as well as, to the efficiency of AAS with recirculated NH_3. Finally, the AD at higher *OLR* from the one tested in lab-scale should be investigated in order to assess the stability of the process.

Supplementary Materials: The following are available online at https://www.mdpi.com/1996-1073/14/3/787/s1, Figure S1: BMP tests of optimally AAS-treated manure fibers: (a) Evolution of cumulative CH_4 yield [14]; (b) Hydrolysis constant determination by Equation S1 from BMP test., Figure S2: BMP tests of optimally NP manure fibers: (a) Evolution of cumulative CH_4 yield of NP manure fibers [14]; (b) Hydrolysis constant determination by Equation S1 from BMP test., Table S1: Ultimate CH_4 yield (B_0) and hydrolysis constant (k_h) of NP and AAS manure fibers as resulted from Equation (S1), Table S2: CH_4 yield of NP fibers and AAS fibers (without manure) calculated by

Equation (5), Table S3: Expected CH_4 yield of NP and AAS digesters based on the share of substrate, Table S4: Characteristics of Scenario 1 (manure mono-digestion) and Scenario 2 (AD of manure and NP fibers), Table S5: Increase of CH_4 yield achieved from BMPs of digested manure fibers for evaluating the efficiency of AAS at low temperature and with the addition of centrifuged effluent.

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