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Research article

Effects of different treatments of manure on mitigating methane emissions during storage and preserving the methane potential for anaerobic digestion

Sonja Sí Olafsdóttir, Claus Dalsgaard Jensen, Anna Lymeratou, Ulrik Birk Henriksen, Hariklia N. Gavala

Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søltofts Plads 228A, Kgs.Lyngby, 2800, Denmark

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ABSTRACT

Current agricultural practices in regards to storage of manure come with a significant GHG contribution, due, to a big extent, to CH₄ emissions. For example, in Denmark, the agricultural sector is responsible for about 11.1 metric tons of CO₂ equivalents; only about 0.2 metric tons come directly from CO₂, while 6.0 tons come from CH₄. The present study aims at evaluating and comparing two methods based on their effect on suppressing CH₄ emissions during storage as well as on preserving and enhancing CH₄ yield in a subsequent anaerobic digestion step: the commonly applied acidification with H₂SO₄ as acidifying agent and thermal treatment at the mild temperatures of 70 and 90 °C (pasteurization). Although both treatments effectively suppressed CH₄ emissions during storage, they exhibited a significant difference in preserving and/or enhancing the CH₄ potential of manure. Specifically, thermal treatment resulted in 16–35% enhancement of CH₄ potential, while acidification resulted in decreasing the CH₄ yield by 6–23% compared to non-treated manure. Further investigation showed that storage itself positively affected the CH₄ potential of treated manure in a subsequent anaerobic digestion step; this was attributed to microbial activity other than biomethanation during storage. In overall and based on the results obtained regarding suppression of CH₄ emissions during storage as well as CH₄ potential enhancement, pasteurization at the temperatures tested is a promising alternative to the broadly applied acidification of manure.

1. Introduction

Swine manure produced on farms is a valuable resource, as it can be used for energy production, mainly in the form of methane (CH₄), and as a crop fertilizer due to the high nutrient content, particularly nitrogen (N) and phosphorous (P). Yet, it can also impose environmental and social challenges if proper management is not applied and therefore good practices and alternative solutions, which promote sustainability and limit associated challenges have come in the forefront. Among various strategies that differ from country to country, (co)composting, anaerobic (co)digestion and recycling of nutrients via field application are among the most investigated ones (Awasti et al., 2022; Khoshnevisan et al., 2021; Li et al., 2020).

Spreading of manure on the fields can take place during some months before harvesting, i.e. in Denmark it usually takes place from February until harvest in the summer (“Landbrug og Fødevarer - Gylle,” 2018). Manure is therefore collected from the stalls throughout the year and then stored as a slurry to be used as fertilizer in the growing season. In order to minimize the loss of nutrients prior to application, manure storage takes place in tanks as a mixture of solid manure, urine, water, and other constituents, such as sand, straw and bedding. However, the storage of manure presents certain challenges. The N content of manure, originating from urine and proteins, is susceptible to losses through its transformation to ammonia (NH₃), nitrous oxide (N₂O), nitrogen oxides (NOₓ) and nitrate, NO₃. Ammonia can be hazardous to the health of humans and animals, and soluble N leaks can cause a nutrient imbalance in nearby soil and water supply as well as inducing acidification and eutrophication problems (NRC, 2003; Schlesinger, 1991; vanBreemen et al., 1982). Storage containers equipped with a floating top layer are usually applied in order to substantially reduce emissions of ammonia; however, they have been found to increase emissions of other gases, such as CH₄, carbon dioxide (CO₂) and N₂O (VanderZaag and Gordon, 2003).
2008). It is mainly anaerobic degradation of organic material in manure that results in the emission of CH4, which in turn traps heat in the atmosphere, contributing to global warming (CH4 actually has a global warming potential 21 times higher than CO2) and climate change (Oonk et al., 2015).

Agriculture is the second most polluting sector in Denmark, behind only the energy sector and mostly due to the large amounts of CH4 emissions. In fact, the agricultural sector is responsible for about 11.1 metric tons of CO2 equivalents, a roughly 20% share of the country’s total emissions. Only about 0.2 metric tons come directly from CO2, while 4.8 metric tons and 6.0 tons come from N2O and CH4 respectively (UNFCC, 2021) (Fig. S1 in Supplementary material). Reducing CH4 emissions from agriculture would count significantly towards decreasing Denmark’s total contribution to greenhouse gas emissions.

To minimize the emission of greenhouse gases and NH3, various technologies have been developed that can be used to treat manure before and during storage. These comprise of cooling, frequent cleaning of the stables and cleaning of the air by using chemical treatment in the ventilation. Another type of treatment that is becoming increasingly common is acidification (Ungpese Poged, 2012). When applied to manure, as has commonly been done in Denmark since 2010, acidification has the well-documented effect of decreasing emissions of NH3 and consequently improving the fertilizer quality (Fangueiro et al., 2015b, 2015a). Since 80% of total NH3 emissions come from livestock manure (Eriksen et al., 2012), a reduction in the NH3 emissions of manure can have significant effects on protecting the environment. Furthermore, previous studies have indicated that acidification may also be effective at reducing CH4 emissions during storage (Petersen et al., 2012; Sokolov et al., 2019; Vechi et al., 2022), while sulfuric acid has been reported as more effective in suppressing CH4 emissions compared to nitric and phosphoric acid as well as organic acids (Dalby et al., 2022). In Denmark, approximately 20% of all animal slurry is treated with sulfuric acid (H2SO4), bringing the pH down to 6.0 or lower, usually around 5.5 (Jensen et al., 2018). Reducing the pH of the slurry causes the equilibrium between NH4 and NH3 to shift towards NH3, thereby abating N loss to the air in the form of NH3 (Fangueiro et al., 2015a). Studies have also shown that chemical hydrolysis may be more accelerated compared to enzymatic hydrolysis at lower pH, while H2SO4 addition has been indicated to decelerate all the other microbial pathways of anaerobic digestion (Fangueiro et al., 2015a). Therefore, as it decelerates methanogenesis as well, CH4 emissions should be reduced by long-term acidification. In addition, it has been reported that acidification reduces the CO2 emission during storage, while hydrogen sulfide (H2S) emissions have been reported to either decrease or be unaffected by acidification (Fangueiro et al., 2015a).

The main limiting factor of the acidification technology is the handling of concentrated acid and safety measurements that need to be taken, eventually resulting into an increased cost. Another limiting factor is the biogas production in anaerobic digestion from manure acidified with H2SO4, which has been reported to be lower than that of non-acidified manure. This seems to be due to either the competition for the substrates between sulfate reducing bacteria (SRB) and CH4 formers and/or the toxicity due to sulfide, causing a poor methanogenesis (Chen et al., 2008; Karhadkar et al., 1987). Therefore, treatment with H2SO4 although suppresses CH4 emissions during storage, it may be detrimental to the final CH4 yield during anaerobic digestion, where maximizing CH4 production is the objective. Thus, alternative solutions would be of interest.

Another alternative practice to acidification, reported an mild heat treatment similar to pasteurization could be very effective in both suppressing methanogenesis during storage but also enhancing CH4 yield during anaerobic digestion. Pasteurization is traditionally used to suppress microbial activity, which is why it might be useful in suppressing CH4 emissions. Zhang et al. (2020) further showed that pasteurization can increase the CH4 yield of some specific substrates during anaerobic digestion. Furthermore, it can relatively easily be applied on pig farms or in biogas plants, especially if coupled with a manure heat exchanger system, as described in two pending patents (Henriksen et al., 2021a; 2021b). In short, the cold manure (20 °C) is pumped through a heat exchanger and heated to 70 °C, the warm manure is transferred into a buffer tank, where it is additionally heated to 80 °C by an external heat source (e.g. electricity or steam). After that, the warm manure is pumped back into the same heat exchanger, to warm up the cold manure and leaves the heat exchanger at 30 °C. Therefore, the only energy needed is the temperature difference of 10 °C, which gives a low energy consumption (Fig. S2 in supplementary material).

Thermal treatment of sludge at low temperatures (70–90 °C) has been extensively investigated so far as a means to remove pathogens (Skidas et al., 2005), increase dewaterability (Liu et al., 2019) and enhance CH4 yield in a subsequent anaerobic digestion step (Appels et al., 2015; Climent et al., 2007; Ferrer et al., 2008; Kim et al., 2022; Lu et al., 2008) with very promising results. Application of thermal treatment on manure and manure fibers with focus on the enhancement of CH4 yield (Rafiaque et al., 2010; Vergote et al., 2020) has also shown application potential, especially if coupled with a manure heat exchanger system as described above.

The scope and the novelty of the present study is to compare acidification with H2SO4 and mild thermal pre-treatment as storage practices for swine manure with focus on methane emissions mitigation during the storage period and how a subsequent, anaerobic digestion step is affected in regards to methane potential. Thermal treatment has been mostly studied in respect to enhancing methane potential while methane emissions mitigation has been investigated mainly through acid treatment; in the present work we directly compare both methods in respect to both mitigating methane emissions during storage as well as to maintaining/enhancing methane production in a subsequent AD step. Therefore, research focused on how a mild thermal pre-treatment (pasteurization at 70–90 °C to allow the use of heat exchangers as described above) of swine manure of short duration (30–90 min) prior to storage affects both CH4 emissions during storage and CH4 production in subsequent anaerobic digestion. The effect of acidification of swine manure with H2SO4 was also investigated at pH values close to the industrial standard (5.5). Besides monitoring the methane production and in order to distinguishing methanogenic and other than methanogenic microbial activity, biological activity was also evaluated via other indicators, i.e. VFA and CO2 generation.

2. Materials and methods

2.1. Feedstock and inoculum

The swine manure used for all experiments was collected at Villads Sørensen farm located in Hastrup in February 2021. On the farm, the manure runs from the stables to a buffer tank from where it is pumped to a large long-term storage silo approximately once a week. The manure used in these experiments was collected from the buffer tank. Prior to any further treatment, the swine manure was stored outside at cool temperatures in sealed containers for one day. After treatment (pasteurization or acidification), all samples - including a control sample - were kept at 4 °C for 3 days, after which a portion of the samples was used to set up storage experiments and biochemical methane potential (BMP) tests as described in subsequent sections. The remaining samples were kept in closed containers at –20 °C.

The inoculum used for the experiments originated from a 3 L lab-scale continuous stirred tank reactor (CSTR) type digester, operating on manure at mesophilic conditions at DTU Chemical Engineering. The inoculum had a pH of 8.65 and Total (TS) and Volatile (VS) Solid content of 1.79 ± 0.02% and 0.78 ± 0.03% respectively.

2.2. Pasteurization and acidification treatments

Pasteurization of the swine manure was carried out under four set of conditions, at two different temperatures, 70 and 90 °C and two
durations, 30 and 90 min 1 L of each sample was put in an Erlenmeyer flask, equipped with a thermometer and a magnet for stirring, and placed on a heated stirring plate. The sample temperature was gradually raised to either 70 or 90 °C and then maintained at the respective temperature for either 30 or 90 min.

For acidification of the swine manure, 0.5 L of manure was put in a glass container, equipped with a magnet and placed on a stirring plate. Then, the pH of the swine manure was adjusted to 5.0, 5.5 and 6.0 respectively by addition of H₂SO₄ (95% purity). This was done slowly in order to minimize foam formation and subsequently the acidified samples were transferred to 1L blue-cap flasks. Prior to performing BMP tests, the pH of the acidified samples was adjusted to the pH level of the swine manure (7.8) and pasteurized samples with addition of KOH (after inoculum addition) so comparison of the CH₄ yields among the different tests was possible. When the BMPs were set up for the acidified samples after storage, the pH level was again adjusted to pH 7.8 (after the addition of the inoculum).

2.3. Storage tests

All samples - raw, pasteurized and acidified manure were put in storage at two different temperature conditions: 4 °C and room temperature. This was done in order to quantify the difference between laboratory conditions (room temperature) and conditions that more closely resemble the real cold conditions for storage of manure in Denmark. Each sample was set up in triplicate and prepared by adding 50 mL of the sample into a 110 mL flask. The flasks were flushed with N₂ for 5 min, immediately sealed with rubber stoppers and aluminium crimps and stored for 78 day without agitation or mixing. During the storage period, the amount of produced gas and its composition were determined, 31 and stored for 78 day without agitation or mixing. During the storage period, the amount of produced gas and its composition were determined.

2.4. Biochemical methane potential, BMP, tests

All BMP tests were performed by filling 320 mL infusion bottles with 12 mL of sample and 100 mL of inoculum, corresponding to an inoculum addition) so comparison of the CH₄ yields among the different tests was possible. When the BMPs were set up for the acidified samples after storage, the pH level was again adjusted to pH 7.8 (after the addition of the inoculum).

To evaluate if a possible enhancement in the methane potential of the pasteurized samples originates from the solid or the liquid fraction, the performance of the solid fraction of a pasteurized manure condition (70 °C, 90 min) was compared to that of the solid fraction of untreated swine manure (control sample) in a BMP test. To perform the separation into solid and liquid fractions, samples were passed through a 2.7 µm glass microfiber filter in a Büchner funnel and flask connected to a hydraulic vacuum pump. After the liquid had passed through the filter the remaining solids were washed with millipore grade water to further remove all soluble material. A sample of the obtained pasteurized and control solids were analyzed for Total and Volatile solids before setting up the BMP tests. When setting up the BMP of the solid fractions, the same amount of solids that were present in 12 mL of a manure sample were combined with the same amount of inoculum used in other BMP experiments (100 mL).

2.5. Analytical methods

TS, VS and ash content were determined according to standard methods (APHA et al., 1975). CH₄ and CO₂ determination was carried out by Gas Chromatography (GC) with a Thermal Conductivity Detector (GC8222, Mikrolab Aarhus, Denmark). The GC was equipped with a Porapak Q packed column (6 ft. and I.D. 3 mm) and N₂ was used as a carrier gas. Injection, oven and detector temperature was set at 70 °C. Quantification of Volatile Fatty Acids, VFA, was done by means of an HPLC (Shimadzu, USA) equipped with a refractive index detector and an AMINEX HPX-87H (Bio-Rad) column at 63 °C. A solution of 12 mM H₂SO₄ was used as eluent at a flow rate of 0.6 mL/min. The samples for the HPLC were centrifuged at 10,000 rpm for 10 min, filtered through 0.45 µm, acidified with H₂SO₄ (10% w/w), centrifuged at 10,000 rpm for 10 min and filtered through 0.20 µm.

2.6. Data processing methods

The kinetics of the CH₄ production from the solid fraction of untreated and pasteurized manure was compared by fitting the data to a 1st order kinetic model following the assumption that hydrolysis is the limitation step of biomass AD (Jensen et al., 2011; Lymeratou et al., 2021):

\[ B(t) = B_0 \cdot (1 - \exp(-kt)) \]

where \(B(t)\) is the CH₄ yield in mL/gVS after \(t\) days of digestion, \(B_0\) is the ultimate CH₄ yield in mL/gVS, \(k\) is the hydrolysis rate in d⁻¹ and \(t\) is the time in d. The predicted CH₄ production curves from the 1st order models were plotted against the experimentally produced CH₄ production curves.

To test whether measured differences between treatments were statistically significant, an Analysis of Variance (ANOVA) test was performed on the final measurements of accumulated CH₄ production levels. For the acidified samples, the analysis was performed using one-way ANOVA, as these treatments differ only in one variable (pH level), whereas the pasteurized samples were compared using two-way ANOVA, as the pasteurized treatments differ in two variables (time and temperature). When different treatments are compared to each other (acidified vs. pasteurized) or when pasteurized treatments are compared to the control treatment, the analysis was performed by using one-way ANOVA. Where the ANOVA test revealed significant differences, a test was subsequently used to assess the treatments that were statistically different from one another.

2.7. Experimental set-up

In order to evaluate the effect of the treatments on the CH₄ emissions during storage and CH₄ potential through BMP tests, a fraction of the differently treated swine manure samples and control samples were stored for a duration of 78 days and monitored for the production of CH₄ and CO₂. BMP tests of all samples were performed prior to storage and after storage in order to evaluate the effect of storage on the CH₄ potential. Table 1 provides an overview of the experiments performed in the present study.

3. Results and discussion

3.1. Storage tests

3.1.1. Methane emissions during storage at room temperature

Fig. 1a shows the methane emissions accumulated during storage of samples at room temperature for 78 days. It is evident that both pasteurization and acidification treatments are effective in respect to reducing CH₄ emissions as compared to the control samples. While the accumulated CH₄ emissions from the control sample reached 95 mL CH₄/g VS after 78 days, the acidified sample that emitted the most (pH 6.0) only emitted 6 mL CH₄/g VS, a reduction of 94%. The acidified sample with the least emissions produced no CH₄ at all (pH 5.0), i.e. a 100% reduction. On the other hand, the pasteurized sample that emitted the most (70 °C, 30 min) reached 4.4 mL CH₄/g VS, while the sample
with the least emissions (90 °C, 30 min) emitted 0.2 mL CH₄/g VS, a reduction of between 95% and 99.7%. One-way ANOVA and t-tests between the individual treatments showed that all differences were statistically significant (p-value \(= 2 \times 10^{-5}\) for the one-way ANOVA). In regards to the pasteurized treatments, the samples treated at 90 °C for 30 and 90 min have emitted only 0.2 and 0.3 mL/g VS respectively, which is only slightly higher than the acidified sample with pH 5.0 that emitted no CH₄. Furthermore, the biogas production potential may be higher for the thermally treated samples as discussed in the section with results from BMP tests. As the treatments objective is not only to reduce emissions but also to enhance the CH₄ potential, each treatment method must be evaluated with respect to both factors. The samples treated at 70 °C for 30 and 90 min gave slightly higher emissions than the samples pasteurized at 90 °C, yielding emissions of 4.4 mL/g VS and 2.2 mL/g VS respectively. A two-way ANOVA test performed on the pasteurized samples rejected the null hypothesis that all samples had the same accumulated CH₄ emissions after 78 days. Both, duration and temperature, have a statistically significant effect on CH₄ emissions (p-values < 0.01). Furthermore, a statistically significant interaction effect (p-value \(= 0.001\)) showed that the intensity of the effect of duration on the CH₄ emissions is dependent on the temperature. For example, when samples were pasteurized at 90 °C the difference of CH₄ emissions between 30 min and 90 min pasteurization is small and when they are pasteurized at 70 °C, the difference between 30 min and 90 min becomes larger.

Comparing the lag phases of the acidified and the pasteurized samples, the samples that have been acidified to pH 6.0 and 5.5 begin emitting CH₄ after only a short amount of time in storage. This is more visible for the pH 6.0 sample, which rises sharply at the beginning of the period, and where emissions are still increasing after 78 days in storage. Pasterurized samples begin emitting CH₄ later, starting at around day 20.

### 3.1.2. Methane emissions during storage at cold conditions

In real-world applications, manure is not always stored at room temperature. For example in Denmark, manure is most often stored in large containers, often kept outside, where the temperature is cooler (Lyngsø Foged, 2012). An identical set of samples as the room temperature samples was therefore stored at 4 °C for the same period of time and measured at the same interval to assess the effect of the colder temperature on emissions during storage. Fig. 1b shows the accumulated CH₄ emissions for the set of samples stored at 4 °C.

Methane emissions were significantly lower overall at 4 °C than at room temperature; after 78 days in storage, the control sample had accumulated 8.8 mL CH₄/g VS, compared to 95 mL/g VS at room temperature. The accumulated emissions of the treated samples have decreased as well; for example, the accumulated CH₄ emissions of the pH 6.0 sample after 78 days reached just 1.7 mL/g VS, compared to 6.1 mL/g VS at room temperature.

The pasteurized samples produced no CH₄ emissions at all during the storage period, and neither did the pH 5.0 acidified sample. The only samples producing small amounts of CH₄ during the storage period were the pH 5.5 sample and the pH 6.0 sample, emitting 0.4 and 1.7 mL CH₄/g VS respectively. The differences between different treatments was again tested using one-way and two-way ANOVA. For the acidified treatments, results were similar to the ones for room temperature results, where a one-way ANOVA rejected the null hypothesis that all treatments had the same average methane production after 78 days (p-value \(= 3 \times 10^{-5}\)). The t-tests between the individual treatments supported the conclusion that pH 6.0 produced more CH₄ than pH 5.5, which further produced more CH₄ than 5.0. For the pasteurized treatments, on the other hand, the results at 4 °C differed somewhat from the results at room temperature. At 4 °C, only temperature was significant in determining CH₄ emissions (p-value \(= 7 \times 10^{-7}\) while time is insignificant (p-value \(= 0.21 > 0.01\)). It seems therefore that although an increase in temperature from 70 °C to 90 °C during pasteurization could lower CH₄ emissions of swine manure, there would be no added benefit to increase the time from 30 min to 90 min, reducing this way the

### Table 1

Overview of the experiments performed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment conditions</th>
<th>Storage, 78 d at room T</th>
<th>Storage, 78 d at 4 °C</th>
<th>Prior to storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurization</td>
<td>70 °C, 30 min</td>
<td>CH₄ emissions, BMP</td>
<td>CH₄ emissions, BMP</td>
<td>BMP and solid fraction</td>
</tr>
<tr>
<td>Acidification</td>
<td>pH 5.0</td>
<td>CH₄ emissions, BMP</td>
<td>CH₄ emissions, BMP</td>
<td>BMP</td>
</tr>
<tr>
<td>Acidification</td>
<td>pH 5.5</td>
<td>CH₄ emissions, BMP</td>
<td>CH₄ emissions, BMP</td>
<td>BMP</td>
</tr>
<tr>
<td>Control (no treatment)</td>
<td>pH 6.0</td>
<td>CH₄ emissions, BMP</td>
<td>BMP and solid fraction</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Methane emissions during storage at room temperature (a) and at 4 °C (b).
heating requirements of pasteurization.

Overall, all treatments studied were found to be effective at suppressing emissions of CH\textsubscript{4} during storage with pasteurization treatments being more effective at the colder storage temperature. Table 2 presents the reduction in emissions compared to the control samples and shows that while the relative reduction in emissions increased for the pasteurized samples, it decreased for the acidified samples. As 4 °C storage temperature can be considered as more representative of usual Danish storage conditions, the obtained results are certainly relevant for farm application in cold regions.

The decrease of CH\textsubscript{4} production activity as a result of acidification is in line with previous studies (Ottosen et al., 2009; Petersen et al., 2012; Shin et al., 2019). Most studies agree that acidification affects CH\textsubscript{4} production through its effect on methanogens and the process of methanogenesis. However, the evidence is not conclusive on the underlying mechanism. Shin et al. performed microbial analysis on acidified manure samples, which revealed that with decreasing pH, the share of the methanogen \textit{Methanosarcina flavescent}, which is known to have both acetoclastic and hydrogenotrophic CH\textsubscript{4}-producing pathways, decreased, while the share of only hydrogenotrophic methanogens increased (Shin et al., 2019). Petersen et al., who were among the first to study the effects of H\textsubscript{2}SO\textsubscript{4} on methane emissions, presented two theories for why H\textsubscript{2}SO\textsubscript{4} could affect CH\textsubscript{4} production; first, the lower pH level itself, and second, the toxicity of the added sulfate, which both inhibit the activity of methanogens in the manure. In other words, even at relatively high pH levels, it is still possible to reduce CH\textsubscript{4} emissions due to the toxicity of the sulfate (Petersen et al., 2012). Sulfate acts as an external electron donor for sulfate reducing microorganisms, which directly compete with acetoclastic methanogens. The product of sulfate reduction is H\textsubscript{2}S, which is also a known inhibitor of methanogens (Chen et al., 2008; Karhadkar et al., 1987). Therefore, the question is whether treatment with H\textsubscript{2}SO\textsubscript{4} for suppressing CH\textsubscript{4} emissions during storage allows for an efficient anaerobic digestion in a subsequent step, even after neutralization.

Other studies have evaluated the effect of storage temperature on the CH\textsubscript{4} emissions. Im et al. studied the effect of storage temperature on the CH\textsubscript{4} emissions from cattle manure and found that the share of psychrophilic methanogens increased with lower storage temperature. At 4 °C there is likely very little methanogen activity anyway, but they find that even at higher temperatures (around 15 °C), CH\textsubscript{4} emissions are lower because psychrophilic methanogens, which have a lower CH\textsubscript{4} production rate than mesophilic methanogens, constitute a higher share (Im et al., 2020).

Analyzing CO\textsubscript{2} emissions during storage at both room temperature and cold conditions (Table 3), one can see that pasteurization at 90 °C was by far more efficient in suppressing CO\textsubscript{2} emissions compared to acidification, especially compared to the higher pH values of 5.5 and 6.0. Thus, a low pH value is detrimental to methanogens but can support other kind of microbial activity. This can be acceptable during storage, though only if the degraded material is conserved in the tank and facilitates the subsequent CH\textsubscript{4} formation during anaerobic digestion. On the other hand, if the degradation during storage results into C loss prior to anaerobic digestion, which is likely to occur, it is highly undesirable.

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Room ( T \text{ mL} )</th>
<th>% decrease against control</th>
<th>( 4 \text{ °C mL} )</th>
<th>% decrease against control</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 °C, 30 min</td>
<td>4.4</td>
<td>95.4</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>70 °C, 90 min</td>
<td>2.2</td>
<td>97.7</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>90 °C, 30 min</td>
<td>0.2</td>
<td>99.8</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>90 °C, 90 min</td>
<td>0.3</td>
<td>99.7</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>0.0</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>2.0</td>
<td>97.9</td>
<td>0.4</td>
<td>95.5</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>6.1</td>
<td>93.4</td>
<td>1.7</td>
<td>80.4</td>
</tr>
<tr>
<td>Control</td>
<td>95.1</td>
<td>8.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The BMP tests before and after storage can indicate whether the non-methanogenic activity during storage could be beneficial or not.

### 3.2. Biochemical methane potential, BMP, tests

#### 3.2.1. Prior to storage

Fig. 2 shows the BMP of each treated sample prior to storage as well as the BMP during storage. Fig. 2a shows the BMP of pasteurized samples prior to storage, and Fig. 2b shows the BMP of acidified samples prior to storage. Table 2 presents the BMP results of each treated sample prior to storage.

#### Table 2

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Room ( T \text{ mL} )</th>
<th>% decrease against control</th>
<th>( 4 \text{ °C mL} )</th>
<th>% decrease against control</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 °C, 30 min</td>
<td>18.9</td>
<td>81.3</td>
<td>1.7</td>
<td>89.3</td>
</tr>
<tr>
<td>70 °C, 90 min</td>
<td>11.0</td>
<td>89.1</td>
<td>0.5</td>
<td>96.9</td>
</tr>
<tr>
<td>90 °C, 30 min</td>
<td>1.2</td>
<td>98.8</td>
<td>0.1</td>
<td>99.4</td>
</tr>
<tr>
<td>90 °C, 90 min</td>
<td>1.3</td>
<td>98.7</td>
<td>0.1</td>
<td>99.4</td>
</tr>
<tr>
<td>pH 5.0</td>
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<td>Control</td>
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<td>16.0</td>
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### Fig. 2. BMP of pasteurized (a) and acidified samples (b).
Acidified samples (Fig. 2a) produced considerably less CH₄ compared to the control sample during anaerobic digestion. Specifically, samples with a pH of 5.0 have accumulated 302 mL CH₄/g VS, compared to 316 mL/g VS for pH 5.5 and 368 mL/g VS for pH 6.0 while the control sample produced 470 mL/g VS. Since the pH levels were adjusted before starting-up the BMP tests, any difference between the acidification treatments are attributed to the inhibition effects of the added sulfate rather than the difference in pH, as discussed earlier. On the other hand, the pasteurized samples (Fig. 2b), produced slightly more CH₄ than the control sample. Specifically, the samples treated at 70 °C for 30 min produced around 535 mL/g VS and the samples treated at 70 °C for 90 min had a slightly lower production of 521 mL/g VS, while the samples treated at 90 °C had produced 495 and 505 for 30 and 90 min respectively. In order to conclude on the statistical significance of these differences, a one-way ANOVA test was run on the acidified samples, while a two-way ANOVA was used on the pasteurized samples. When the acidified treatments were compared with each other, the ANOVA test yielded a p-value of 0.32, which is higher than the chosen significance level of 0.05, so it is not possible to reject the null hypothesis that all acidification treatments have the same average level of methane production. When compared to the control sample a p-value of 0.006 was obtained, indicating that even though the acidification treatments are not statistically different to each other, they all have significantly less CH₄ production than the control sample.

The two-way ANOVA test for the pasteurized samples yielded a p-value of 0.92 for time and 0.19 for temperature. Since both p-values are higher than the significance level 0.05, the null hypothesis that all pasteurized samples have the same average methane production cannot be rejected. Furthermore, when the control sample was included, the ANOVA test yielded a p-value of 0.17, meaning that the pasteurized manure samples statistically produced the same amount of CH₄, indicating no negative effects on the subsequent anaerobic digestion process when manure is pasteurized, in contrast to the acidified samples.

According to the discussion based on literature studies in the previous section, it was not surprising that the acidified samples showed considerable decreased methane yields during the methane potential tests. In regards to the pasteurized samples, Hu et al. (2015) have applied thermal treatment at 70 °C on high-solid content swine manure for 1–4 days and have observed an average of 17% increase of the methane potential compared to the control, non-treated manure. This is well in-line with the results obtained in the present study, which showed an average of 9% increase at much lower treatment durations. On the other hand, Carrere et al. (2009) applied thermal treatment at 70 and 90 °C on the liquid and total fraction of manure for 3 h and concluded that the methane potential of liquid phase was enhanced by 70 and 89% after a treatment at 70 and 90 °C respectively, while the total fraction generated 13% higher and 12% lower methane yields when treated at 90 and 70 °C, respectively. Again, the longer duration, could justify the slightly higher methane yield obtained with the total fraction of manure treated compared to our study at 90 °C. The authors did not comment on the lower methane yield obtained with the total fraction after 70 °C though and this is in contradiction to our results, which showed a slightly higher increase of methane yield even at 70 °C. Bonmati et al. (Bonmati et al., 2001) also observed 53% enhancement of methane yield at BMP tests at a neutral starting pH of 7.2 (comparable to this study) when pig slurry was pre-treated at 80 °C for 3h. Again the higher increase compared to our study could be due to longer duration and/or different feedstock characteristics. Raju et al. (2013) reported a 24% enhancement of methane yield when pig manure was pre-treated at 100°C for 15 min. In overall, our findings are in agreement with most literature studies in respect to the positive effect of thermal pre-treatment to the anaerobic digestion of swine manure – variations in % enhancement are anticipated due to differences in treatment conditions, inoculum and feedstock differences.

3.2.2. After storage at room temperature

Fig. 3 shows the BMP of each treated sample after storage for 78 days at room temperature. The pasteurized samples (Fig. 3a) produced more CH₄ after storage compared to the control sample, while the CH₄ production of the acidified samples (Fig. 3b) was very similar to the control sample. Specifically, the acidified treatments yielded an accumulated CH₄ production of between 426 mL/g VS and 544 mL/g VS, while the pasteurized treatments produced between 676 mL/g VS and 708 mL/g VS. The control sample showed an accumulated production of 516 mL/g VS. Pasteurization therefore increased CH₄ production by 31–37%. A one-way ANOVA test showed that all pasteurized samples were statistically significantly different to the control sample (p-value of 0.0001 < 0.01). However, when the pasteurized treatments were tested among themselves with a two-way ANOVA test, neither time nor temperature created a statistically significant difference between the different pasteurization treatments (p-values for time and temperature were 0.39 and 0.51 respectively). When the acidified samples and the control sample were compared, there was a statistically significant difference between at least one of the treatments and the control sample (p-value of 0.0004 < 0.01). Pairwise t-testing, which compared each of the
treatments to each other, indicated that while all of the acidified treatments were significantly different among themselves, only the pH 5.0 treatment was significantly different to the control sample. The storage tests, discussed in the previous section, demonstrated that, to a high degree, the acidified and the pasteurized treatments were both able to preserve organic matter as documented by the lower emissions of methane during storage from the treated samples. The fact that the acidified samples exhibited a lower BMP despite the preservation of organic material can therefore be partially attributed to the inhibition of methanogenic activity. As with the prior-to-storage samples, the pH levels of the acidified samples were adjusted before measuring the BMPs, in order to make comparison possible between BMPs prior to and after storage. The pH levels themselves therefore did not contribute to the difference between the acidification treatment so any difference must be due to the inhibition effects of the added sulfate on the methanogens.

3.2.3. After storage at cold conditions

Fig. 4 shows the BMP of each treatment after storage for 78 days at 4 °C. Pasteurized samples exhibited a higher CH₄ potential compared to control, however, the increase was less intense compared to the pasteurized samples that were stored at room temperature (an average of 16% and 35% increase, respectively). This could be attributed to a higher degree of partial degradation and production of acids of manure during storage at room temperature compared to storage under cold conditions that could facilitate subsequent conversion to CH₄ (see section 3.2.4).

After 37 days, the accumulated CH₄ production of the acidified samples was between 354 and 453 mL/g VS, while for the pasteurized treatments the accumulated production was between 586 and 624 mL/g VS. The control sample, by comparison, presented an accumulated production of 519 mL/g VS. When stored at 4 °C, pasteurization therefore seems to increase the production of CH₄ by between 13.5 and 20.8%, while acidification suppresses the production by 12.2 and 31.3% compared to the control sample.

The results of ANOVA tests were also similar to the results prior to storage and the results after storage at room temperature: The pasteurized samples were significantly higher than the control sample but were not significantly different among themselves. The acidified samples were, however, both significantly different to the control sample and different among themselves, with the exception of pH 5.0 and pH 5.5 where t-test yielded a p-value of 0.12.

3.2.4. BMP of the solid fraction and effect of storage

According to the results of BMP tests, pasteurization enhanced the CH₄ potential after storage. In order to assess at which extent pasteurization affected the hydrolysis rate of the solid matrix, as it has been reported for other treatments as well (Lymperatou et al., 2020, 2021, 2022), BMP experiments of the solid fraction of the control sample and of the solid fraction of one of the pasteurized samples (70 °C, 90 min) were carried out. The experiment was performed using a pasteurized sample and a control sample that had not been stored, in order to isolate the effect of the treatment from any potential effect from the storage. The resulting cumulative CH₄ plots are shown in Fig. 5. Fitting of experimental data with the 1st order kinetic model revealed that, indeed, pasteurization resulted in enhancement of the hydrolysis rate. Hydrolysis constants of 0.08 and 0.11 d⁻¹ were calculated for the control and pasteurized sample (with a regression coefficient R² of 0.97 and 0.96), respectively, which corresponds to an increase of 27% of the hydrolysis rate due to pasteurization.

A one-way ANOVA test showed that for the second to last measurement, taken at day 23, the difference was statistically significant (p-
value of 0.02), but for the last measurement, taken at day 35, the difference between the two solids fraction was insignificant (p-value = 0.11). This implies that the pasteurization only accelerated the rate of hydrolysis but did not promote a significant difference in the total biodegradability and the subsequent CH₄ potential of the solid fraction. This finding is in agreement with the results from BMP tests performed with manure prior to storage (section 3.2.1.), where the ultimate methane yields were comparable.

The above conclusion combined with the observation that storage of pasteurized samples at two different temperatures affected the BMP positively implies that the effect of storage is indeed significant. Although pasteurized samples exhibited very low CH₄ production during storage, the increase of the volatile fatty acids level before and after storage (Fig. 6) revealed that there was indeed microbial activity other than biomethanation in the pasteurized samples.

4. Conclusions

Both pasteurization and acidification effectively suppressed emissions of CH₄ during storage at room temperature and cold conditions (an average of 99 and 95% reduction respectively) with pasteurization being even more effective than acidification at the colder storage temperature. Acidification was less effective in suppressing microbial activity in general, as acidified samples reduced CO₂ emissions by 43–70% compared to 90–99% CO₂ emissions suppression by pasteurization.

In regards to subsequent anaerobic digestion step, pasteurized samples produced significantly higher amounts of CH₄ compared to acidified samples. Specifically, pasteurized samples produced 35 and 16% more CH₄ compared to control samples after storage at room temperature and cold conditions, respectively. On the contrary, acidification resulted in a considerable, 23% decrease of the CH₄ potential compared to control samples after storage at cold conditions, while the CH₄ yield decrease at room temperature was less pronounced (an average of 6% decrease).

Storage itself exhibited also a positive effect to the CH₄ potential of treated samples acting synergistically with the heat application (pasteurization) while soothing the negative effect of the acidification. Specifically, prior to storage, the CH₄ potential of pasteurized samples was just 9% higher compared to the CH₄ yield of non-treated samples, while acidified samples showed an even lower CH₄ potential compared to the non-treated samples (an average of 30% lower CH₄ potential). Further analysis of the volatile fatty acids levels prior to and after storage showed that there was significant microbial activity other than biomethanation in the pasteurized samples, which could render the organic matrix more amenable to biomethanation in the subsequent anaerobic digestion step. The positive effect of pasteurization was found to be due to enhancement of the solids hydrolysis rate, which also enhanced the rate of biomethanation.

Based on the above, it is concluded that pasteurization at the low temperatures tested is a promising alternative to the broadly applied acidification, in regards to both CH₄ emissions suppression during storage as well as CH₄ production enhancement in a subsequent anaerobic digestion step. Validation at farm applications as well as investigating the fate of organic and inorganic nitrogen and phosphorous upon application of thermal treatment is the natural next step of this work.

Credit author statement

Sonja Síf Ólafsdóttir: validation; formal analysis; investigation; writing - original draft, Claus Dalsgaard Jensen: conceptualization; validation; investigation; writing – review and editing, Anna Lympertatou: conceptualization; methodology; writing – review and editing; supervision, Ulrik Birk Henriksen: conceptualization; writing – review and editing; funding acquisition, Harikilia N. Gavala: conceptualization; methodology; resources; writing - original draft; visualization; supervision; funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References
