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Bio-Acidification of Cattle Slurry with Whey Reduces Gaseous Emission during Storage with Positive Effects on Biogas Production

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Abstract: Livestock manure is the main source of ammonia (NH_3) and greenhouse gases emissions (GHG), which can be reduced by acidifying manure. This work assessed the effect of acidification of cattle slurry using whey on NH_3 and GHG emissions during storage, followed by its usage for biogas production. Tests were conducted to optimize the dose and the frequency at which whey was applied to cattle slurry. Two of the analyzed treatments, AS1-100 and AS1-10, showed reduced emissions when compared with the control AS1-0 without whey. In AS1-100, 100% of the optimized amount of whey was added to the slurry at the beginning of the test, while in AS1-10 whey was fractioned in 10 applications (one per day) corresponding to 10% of the total. Batch-type anaerobic digestion assays using AS1-100 and AS1-10 as feedstock resulted in a significant increase in methane production when compared with the anaerobic digestion of AS1-0 (+33% and +53%, respectively). The best results in terms of gas emissions abatement and methane production during anaerobic digestion were obtained when a low organic loading rate of whey was used. These results demonstrate that the use of whey for slurry acidification is a viable approach for potentially solving the economic and environmental problems of GHG and NH_3 emissions during slurry storage, whereby increasing energy and environmental sustainability.

Keywords: ammonia; emission mitigation; food industry byproducts; greenhouse gases; manure management; methane; nitrous oxide; slurry treatment; whey

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1. Introduction

Over the last 150 years, an increment in atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) has been causing a progressive warming of the planet, a phenomenon known as the greenhouse effect. Furthermore, N_2O also substantially contributes to the depletion of the stratospheric ozone layer [1,2]. In 2019, 6.5×10^9 tons of liquid manure and 3.4×10^9 tons of solid manure from dairy cows were produced worldwide [3,4]. The livestock sector contributes 50% and 75% to greenhouses gases (GHG) and ammonia (NH_3) anthropogenic emissions, respectively. Solid manure heaps or stored liquid manure, either in lagoons or in uncovered tanks, are potentially the most dangerous situations for these emissions [5–7]. Ammonia has an indirect impact on the greenhouse effect as it leads to the formation of N_2O once it is emitted into the air [8]. In addition, NH_3 emission is of concern because of its contribution to the eutrophication of watercourses and ecosystems and to water and soil acidification [9]. In manure, NH_3 volatilization appears to be unavoidable and increases with a rise in temperature, pH, and wind or ventilation rate commonly applied in modern livestock buildings [10–12] and drying systems [13].

Several techniques have been tested to improve the management and storage of livestock waste, from both an economic and environmental point of view [14]. A possible approach to achieve a reduction in NH_3 volatilization during storage and field application is slurry acidification [15–19].

Sulfuric acid (H_2SO_4) is a strong acid, and as the cheapest inorganic acid available on the market, it is the most common additive used to decrease slurry pH [19,20]. Nevertheless, besides its corrosiveness and hazards for human and animal health [21], some other constraints exist. Studies have shown that physicochemical changes occur in the slurry after treatment with strong acids [22]. Specifically, carbon could be lost by the protonation of carbonate and subsequent gaseous release as CO_2 [23]. In addition, an inhibition in biogas production is expected when using H_2SO_4 acidified liquid manure to feed biogas plants [24,25].

The decay in biogas yield is likely due to an increase in sulfur (S) content in manure leading to the inhibition of some microorganisms involved in the anaerobic process by sulfate or sulfide [25,26]. In 2021, around 20,000 facilities to produce biogas and biomethane were operational in Europe [27]. Italy ranked second in the EU with approximately 1300 agricultural plants and currently converts 15% of livestock effluents into biogas, with a foreseen share of 65% by 2030 [28]. Thus, while mitigation techniques for GHG and ammonia emission are needed, these should not negatively affect biogas yield.

Organic acids for ammonia emission reduction have been extensively investigated [20,29–32], but their costs call for more economically sustainable treatments. To this extent, easily fermentable biomass can lower the pH of a suspension due to the production of organic acids by endogenous anaerobic microorganisms [31–34]. In 2019, 116.9 million tons of cheese whey were globally produced [4]. Whey is composed of organic material, such as fats, proteins, and lactose, being this carbohydrate the most abundant milk solid that remains in dairy industry wastewater and the most rapidly metabolized [35].

The need to combine environmental and economic benefits is the most widespread demand among dairy farmers who must deal with two problems: GHG and NH_3 emissions during manure storage and whey treatment and disposal. The objective of this work was to investigate whether there is a synergistic effect of mixing manure and whey in reducing the emissions of GHG and NH_3 during storage and in improving biogas production during the anaerobic digestion of bio-acidified manure.

2. Materials and Methods

2.1. Sampling and Chemical Characterization of Dairy Cattle Slurry and Whey

The cattle slurry and whey used in this study were sampled from a commercial dairy farm and cheese factory from the Alto Adige region (northeastern Italy). The pH was measured by a portable pH meter (Hanna Instruments HI 9026) using a glass electrode combined with a thermal automatic compensation system. Total solids (TS) were determined after 24 h at 105 °C and volatile solids (VS) were determined after 4 h at 550 °C in a muffle furnace [36].

2.2. Preliminary Experiments

Preliminary experiments were carried out in order to: (i) define the amount of whey needed to successfully reach the target pH (5.5), and (ii) to assess with what procedure whey has to be added to manure in order to maintain a stable pH value over time.

2.2.1. Phase 1: Assessment of the Amount of Whey Needed for Acidification

Raw cattle slurry was divided into 1 L aliquots into 18 glass jars with a capacity of 2.5 L. Different whey amounts were added afterwards to the slurry to achieve a pH value of 5.5 [16]: 0% (W0, control), 10% (W10), 20% (W20), 40% (W40), 60% (W60), and 80% (W80) calculated on slurry vs. content, equivalent, respectively, to 0, 85.68, 171.37, 342.73, 514.10, and 685.47 g of whey on a wet weight (ww) basis. Acidified slurries (three replicates per treatment) were stored in a temperature-controlled cabinet at 20 °C. The experiment lasted 10 days, which was considered the average on-farm pre-storage period of fresh manure before anaerobic digestion at the biogas plant, in the context of this specific studied area. During the test, three pH measurements spaced apart by 4 h were done on day 1 and 2, two measurements spaced apart by 8 h on the third day, and one measurement for the following days until the end of the experiment.

2.2.2. Phase 2: Assessment of Cattle Slurry Acidification Using Different Frequency of Whey Addition

A dynamic slurry storage experiment (i.e., with the periodic addition of slurry into the storage jars) was designed to assess the acidification process under the slurry storage operating conditions of a typical full-scale livestock farm. Cattle slurry was divided into 1 L aliquots into 24 glass jars with a capacity of 5 L. Slurries were acidified using the optimum concentration of whey (W40) determined during Phase 1 and left undisturbed for the first two days to reach a pH value of approximately 5.5. During the following 10 days of the assay, raw slurry was added daily to the jars in aliquots of 50 mL (AS1) or 100 mL (AS2) to simulate the dynamic conditions of farm-slurry storage. The choice of testing the AS1 scenario was made considering that in a typical medium-sized cattle farm of the study area, it is considered realistic to add a daily volume of slurry equal to 5% of that initially contained in the storage tank; on the other hand, scenario AS2 (i.e., slurry addition equivalent to 10% of that initially contained in the storage tank) provides additional insights on slurry acidification process and optimization. Whey was added to the jars at the optimal dosage (W40) and with different frequencies: (1) AS1-100 and AS2-100: 100% of the calculated amount of whey was added to the slurry at the beginning of the test; (2) AS1-50 and AS2-50: whey was fractioned in two applications, corresponding each to 50% of the total calculated amount (the second whey addition was done 4 days after the first one); (3) AS1-10 and AS2-10: whey was fractioned in 10 applications (one per day) corresponding to 10% of the total. No whey was added to the control samples (AS1-0 and AS2-0). After acidification, slurries were kept in a temperature-controlled cabinet at 20 °C for the whole duration of the experiment. The daily control of pH was carried out as follows: four measurements were performed daily during the first two days of the experiment, and two measurements (before and after the loading of slurry and whey) for the following days until the end of the experiment. The test was carried out with three replicates per treatment. The chemical characteristics of the cattle slurry and whey used in each phase are presented in Table 1.

Table 1. Average chemical characteristics of slurry and whey used in the first and second phases of acidification treatment. In Phase 1, it was defined as the amount of whey needed for optimal acidification (pH 5.5) and in Phase 2, the volume of cattle slurry used daily and the frequency of whey addition to the slurry were defined. TS—total solids; VS—volatile solids.

	Phase 1		**Phase 2**	
	Cattle Slurry	**Whey**	**Cattle Slurry**	**Whey**
TS (%)	6.18	7.77	8.36	7.69
VS (%TS)	84.59	78.52	84.10	78.42
pH	7.10	4.70	6.90	4.60

2.3. *Measurement of Ammonia and GHG Emissions*

Samples AS1-0, AS1-10, and AS1-100 were prepared as described for Phase 2. NH_3 and GHG emission measurements were carried out according to [21,37]. The concentrations of the gases NH_3, CO_2, CH_4, N_2O were measured by the dynamic chamber method [30], using an infrared photo-acoustic analyzer (Multigas Monitor INNOVA 1412, Ballerup, Denmark). Afterwards, nitrous oxide and methane emissions were converted to CO_2 equivalents

(CO_{2eq}) by using the global warming potential values of 265 and 28 for N_2O and CH_4, respectively [38]. The test was carried out in triplicate.

2.4. Biogas Yield of Acidified Slurry

At the end of the emission test, AS1-0, AS1-100, and AS1-10 replicates were collected and used for biochemical methane potential (BMP) tests in batch reactors. The treatments were named AS1-0*, AS1-100*, and AS1-10*, respectively. Each trial was performed in triplicate and was composed of a mixture of the biomasses AS1-0, AS1-100, AS1-10, whey (TS% 7.80 ±0,05; vs. (%TS) 78.59 ± 0.16) and digested slurry used as inoculum (TS% 7.27 ± 0.51; vs. (%TS) 66.83 ± 4.07). The amount of inoculum was calculated so that the ratio of VS from biomass to inoculum was 1:2, according to [39] requirements. Three batch reactors containing inoculum only were used as a control. BMP tests were carried out according to [39,40]. Briefly, 2 L glass digesters sealed with glass gas taps with "L" exit tubes and manually closed valves were used. Each digester was fitted to a Tedlar bag (Tesseraux, Muenchen, Germany) for biogas collection. Trials were carried out under mesophilic conditions (40 ± 2 °C) within a temperature-controlled chamber for a period of 35 days. All batch digesters were manually stirred at least once a day. Samples were analyzed for TS, VS, and pH at the beginning and the end of the BMP test.

The produced biogas volume was measured every 1 or 2 days by using a precision liter counter (Ritter Drum-type Gas Meter type TG05/5, Bochum, Germany), and CH_4 concentration was analyzed by an instrument equipped with infrared sensors (Draeger XAM 7000, Luebeck, Germany). Calculations were performed according to [40].

2.5. Statistical Analysis

The data were subjected to the statistical analysis of variance (ANOVA). The data distribution normality was verified using the Shapiro Wilks test, and the equal variance of different groups was tested using the Levene test. The Bonferroni test was used for the comparison of means among treatments using Rstudio software. A p value lower than 0.05 was considered statistically significant. Pearson's correlation coefficients were calculated using data from GHG emission and methane production during BMP.

3. Results and Discussion

3.1. Preliminary Experiments

3.1.1. Phase 1: Assessment of the Amount of Whey Needed for Acidification

The addition of whey to liquid manure produced a gradual decrease in pH over time (Figure 1). Previous works reported that acidifying slurry to pH 5.5 shifts the equilibrium NH_4^+/NH_3, with 99.8% present as the protonated form [41,42]. The acidification of the mix may be due to the fermentation of lactose present in whey, which is metabolized into organic acids, such as lactic acid, acetic acid, propionic acid, and butyric acid. Whey doses below 40% (samples W10 and W20) were not enough to reach the target pH 5.5. At the end of the test, W40 showed a pH value of 5.7, while lower pH values were observed in W60 and W80 samples. For the control sample (W0), which corresponds to unacidified liquid manure, pH decreased slightly over time, settling around an average value of 6.8. The difference in the slurry pH patterns, which depends on the whey concentration used, could be explained by considering the components that are dominating the buffering capacity of the mix. [43] reported that high concentrations of volatile fatty acids (VFA) or total ammonia determine slurry pH. Therefore, an increment in VFA concentration results in a drop in slurry pH and an inverse behavior is observed when the total ammonia concentration increases. At high whey concentration, the buffer capacity of the slurry is dominated first by VFA and then by the equilibrium NH_4^+/NH_3. Whey carbohydrates are first metabolized, giving rise to VFAs that are responsible for the pH drop observed at the beginning of the experiment. When VFAs are consumed by microorganisms present in the slurry, whey proteins start to hydrolyze producing a raise in NH_3 concentration, thus increasing slurry pH by the end of the test. At the lowest whey concentration (W10), the buffer capacity of the slurry is likely dominated by other components, such as inorganic carbon and phosphorous, that determine a pH trend similar to unacidified slurry.

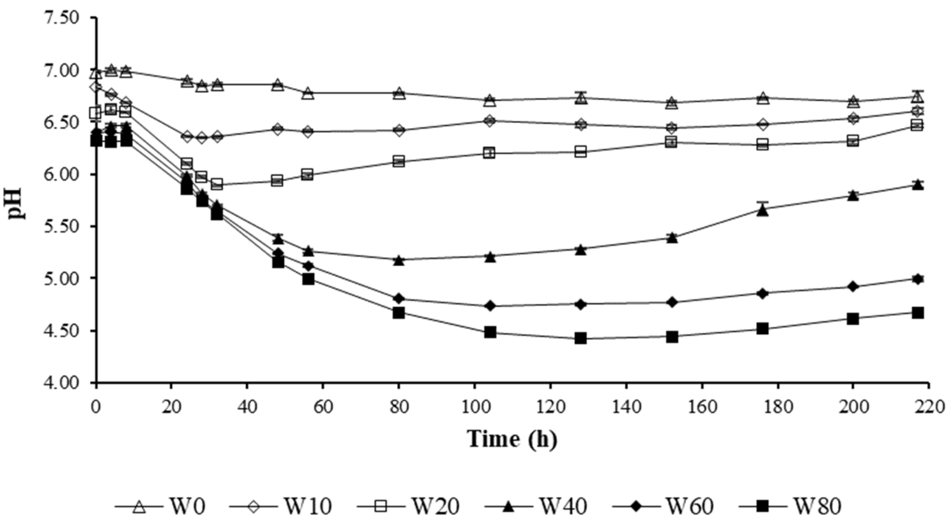

Figure 1. Time course profile of pH values measured during Phase 1 of the test. Error bars indicate the SE ($n = 3$).

Based on these results, the 40% dosage was chosen for the subsequent tests. In addition, W40 was the lowest amount of whey necessary to reach the target pH of 5.5. Indeed, a smaller amount of whey implies a smaller impact on the storage capacity of the farm, on the costs for the construction of storage tanks, and for wastewater management. Another important aspect that must be pointed out is that higher whey dosages (such as W60 and W80) lead to a pH value well below 5 that may be a cause of corrosion to some components of the slurry storage tank.

3.1.2. Phase 2: Assessment of Slurry Acidification under Dynamic Slurry Storage Conditions

The second phase was aimed at assessing the ability of the system to maintain the optimal pH value of 5.5, with a periodic supply of slurry and whey into the storage containers. The test was conducted using the amount of whey corresponding to the concentration that gave the best results in terms of pH reduction (W40) and it comprised two subphases: the static storage subphase that lasted 48 h, and the dynamic storage subphase over the subsequent 10 days.

At the beginning of the test, all trials showed the same pH value except for the controls in which whey was not added (AS1-0 and AS2-0). During the static storage subphase, the acidified slurries presented a drop in pH until an average value close to 5.5 (Figure 2), which confirmed the results obtained in Phase 1. During the dynamic storage subphase, the pH trend showed a different evolution depending on the treatment. Regardless of the volume of liquid manure supplemented daily (AS1 or AS2), a pH value below 5.5 was reached in those trials in which 100% of W40 whey dose was added the first day (AS1-100 and AS2-100) or 10% of W40 whey dose was applied daily during the dynamic phase (AS1-10 and AS2-10) (Figure 2). In the case of AS1-50 and AS2-50 trials, the pH stabilized to an average value of 6 during the whole experiment.

According to these results, for the subsequent emission measurement tests, it was decided to consider the AS1 scenario (representative of a typical cattle farm in the study area) in combination with the two whey loading methods (AS1-10, AS1-100) that provided the best results in terms of pH reduction.

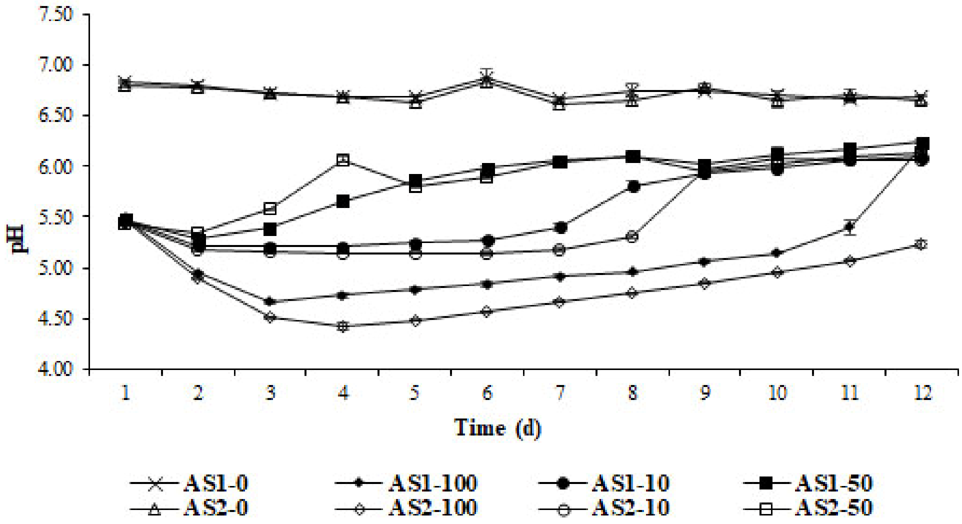

Figure 2. Time course profile of pH values measured during Phase 2 of the test. Error bars indicate the SE (n = 3).

3.2. Ammonia and GHG Emissions from Acidified Slurry

The total emissions of GHG (CH_4, N_2O, and CO_2) and NH_3 were measured using acidified manure from the AS1-10 and AS1-100 trials. AS1-0 was used as a control. Methane emission values were 105.06 ± 17.19 g m^{-2} for AS1-100 and 53.68 ± 5.86 g m^{-2} for AS1-10, which were statistically lower than the control value of 168.20 ± 1.84 g m^{-2} (Table 2). Even though the amount of whey added to each trial was the same, a higher CH_4 emission was detected in AS1-100 in comparison with AS1-10. Ref. [44] demonstrated that at a low organic loading rate of propionic acid production increases in detriment of the decrease in butyric acid, and the opposite occurs at a high organic loading rate. In the case of AS1-10, the addition of whey fractionated in ten doses could have favored the formation of propionic instead of butyric acid, while the opposite could have taken place in AS1-100. [45,46] reported that the propionic acid metabolization rate is the lowest for all the VFAs. This fact could explain the lower amount of CH_4 produced by AS1-10 when compared with AS1-100.

Table 2. ANOVA summary table and Bonferroni's method for multiple comparisons. Total emissions of CH_4, CO_2, and N_2O from acidified slurries and control. Results are presented as mean values, with SE values in brackets (n = 3). Different lowercase letters (a, b, or c) show significant differences among samples ($p < 0.05$).

Sample	CH_4 (g m^{-2})	CO_2 (g m^{-2})	N_2O (g m^{-2})
AS1-0	$168.20_{(1.84)a}$	$1448.80_{(27.63)a}$	$0.23_{(0.03)a}$
AS1-100	$105.06_{(17.19)b}$	$1115.61_{(110.39)a}$	$0.48_{(0.09)a}$
AS1-10	$53.68_{(5.86)c}$	$1245.67_{(90.93)a}$	$0.37_{(0.06)a}$
Degrees of Freedom	2	2	2
Mean Square	9870.9	84,597	0.04887
Pr (>F)	0.00078	0.07916	0.08665
F	29.611	3.987	3.7796

Even though there was no statistical difference in the amount of CO_2 emitted by the samples, AS1-10 and AS1-100 showed a drop in CO_2 emissions of 14% and 23%, respectively, compared with the control (Table 2, Figure A1b). This decrease in emission could be explained by the generation of ammonia during whey protein degradation. In solution, NH_3 molecules react with water to produce hydroxide ions and NH_4^+. Carbon dioxide molecules formed by microorganisms present in the slurry remain partly soluble in water and react with hydroxide ions to form bicarbonate, reducing the fraction of CO_2 that escapes to the gas phase.

Another GHG emission analyzed in acidified samples was nitrous oxide. Comparing acidified slurries with the control sample, there was no statistical difference in the amount of N_2O emissions (Table 2), and this trend could be observed throughout the duration of the emission test (Figure A1c). Nitrous oxide is produced during the nitrification/denitrification process which takes place when aerobic and anaerobic conditions coexist in the slurry [47]. Ref. [48] showed that as O_2 availability in solution decreased, N_2O production from NH_3 oxidation pathways increased and that heterotrophic denitrification was responsible for all N_2O produced at 0% O_2. The slightly higher quantities of N_2O emitted from acidified slurries compared with the control sample could be explained by the oxidation of NH_3 generated during whey protein hydrolysis.

The GHG emissions from the AS1-10 and AS1-100 trials, expressed in CO_2 equivalents, revealed a net total reduction compared with the control sample of 54% and 33%, respectively (Figure 3). A drop in the total GHG emissions observed in acidified slurries was mainly due to the lower CH_4 loss followed by CO_2 and N_2O emissions.

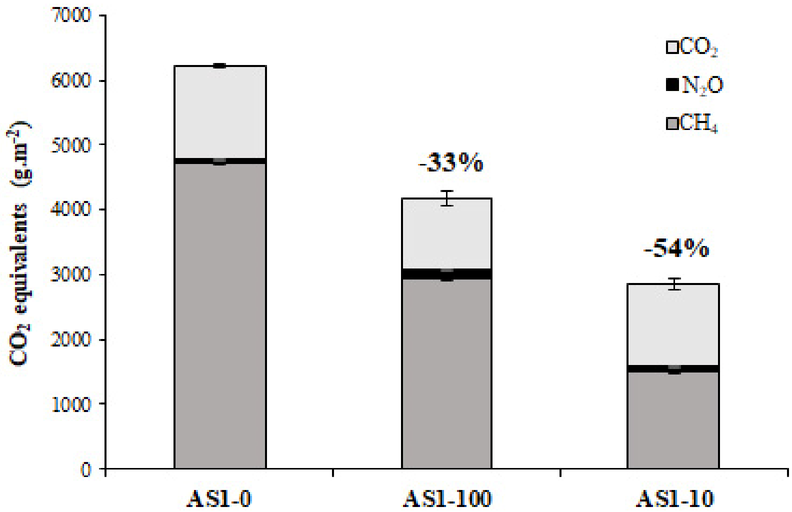

Figure 3. Total GHG emissions expressed as CO_2 equivalents.

With regard to NH_3 emissions, contrasting the amounts emitted from acidified slurries with the control sample exhibited a drop of 90% for AS1-10 and 88% for AS1-100, and there was no statistically significant difference between the acidified slurries (Figure 4a). Furthermore, the analysis of emissions shows that the amount of NH_3 lost from acidified slurries always remained below the value of 1 g NH_3 m^{-2} per day in both pre-load and post-load measurements (Figure 4b). In line with this, [49] reported that when NH_3 emissions were measured in acidified slurries (pH 5.5 and 6.0), the abatement was between 82 and 95% when compared with the control.

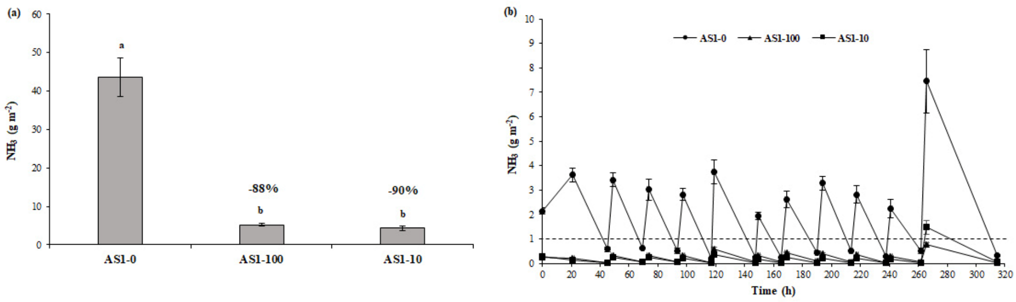

Figure 4. Total emissions of NH_3 (**a**) and time course (**b**) from acidified slurries and control sample. Different lowercase letters (a or b) show significant differences among samples ($p < 0.05$). Error bars indicate the SE ($n = 3$).

3.3. Biogas Yield of Acidified Slurry

The anaerobic digestion of acidified slurries resulted in a significant increase in methane production: +33% (AS1-100*), and +53% (AS1-10*) when compared with the non-acidified slurry (AS1-0*) (Figure 5a). Methane production from AS1-10* (383 ± 3 mL CH_4 g VS^{-1}) was comparable to the methane yield of the whey tested in this study (431 ± 20 mL CH_4 g VS^{-1}, $p < 0.05$) and to the 501 mL CH_4 g VS^{-1} reported by [40]. During the BMP test, the initial and final pH values were closer to neutrality in AS1-10* in comparison with AS1-100*. It is known that methanogen activity is highly sensitive to an abrupt change in pH value, with the optimal range being between 6.8 and 7.5 [50–52]. For this reason, it could be hypothesized that these differences in the pH range between trials could explain the higher methane production observed in AS1-10* compared with AS1-100*. On the other hand, a negative correlation was found between CH_4 emission during storage and CH_4 production during the BMP test (Pearson's correlation coefficient 0.997, $p = 0.051$). Therefore, according to these results, it can be stated that the bio-acidification of slurry reduces methane emission during storage and has no detrimental effects on biogas production during anaerobic digestion.

The daily biogas yield showed a similar trend for the acidified slurries (Figure 5d,e). The biogas production profile was characterized by a peak at the beginning of the test that lasted 4–5 days, and it was followed by a progressive and regular decrease, which dropped to zero after about 35 days. The percentage of methane in the biogas gradually increased up to the greatest value during the first 10 days, and then it stabilized to a constant value. In the cases of AS1-100*, AS1-10*, and whey, the maximum percentage of CH_4 was between 50 and 60%.

At the beginning of the BMP test, AS1-100* and AS1-10* showed a statistically significant difference in TS (%) values in comparison with AS1-0*, but there was no difference among these trials for VS (% TS). By the end of the BMP test, VS (% TS) was lower for AS1-100* compared to AS1-0* (Table 3). On the other hand, the final pH values of the trials were around 7, which is relevant in case the acidified digestate is further used as an organic fertilizer, since it would not significantly change the soil pH from the Italian Alto Adige region [53].

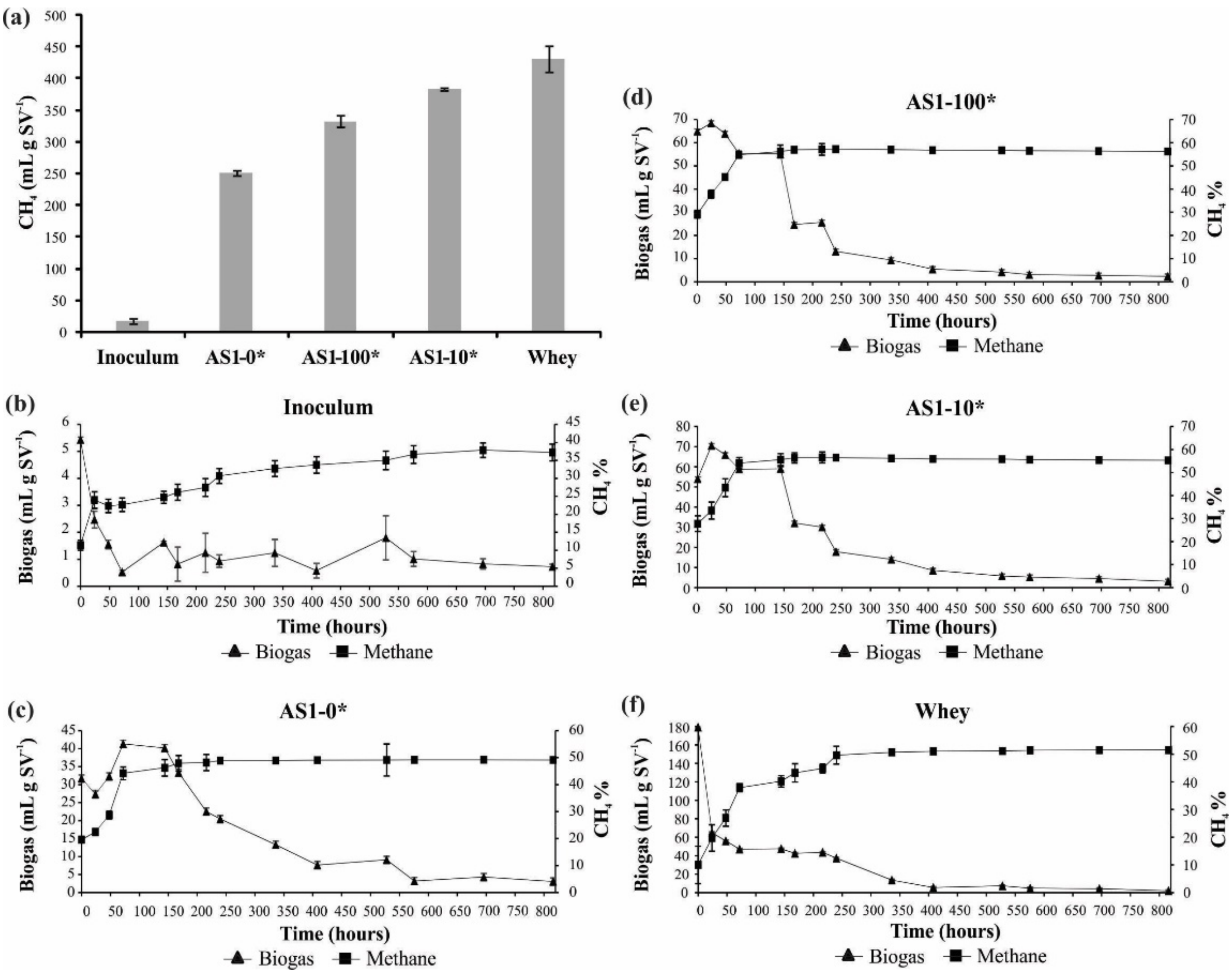

Figure 5. Effect of acidified slurry on biogas production in batch anaerobic digestion tests. (**a**) Total CH_4 production from inoculum, AS1-0*, AS1-100*, AS1-10*, and Whey. Time course profile of biogas and CH_4 production from (**b**) inoculum, (**c**) AS1-0*, (**d**) AS1-100*, (**e**) AS1-10*, and (**f**) Whey. Error bars indicate the sample SE (n = 3).

Table 3. Average chemical characteristics of samples at the beginning (day 0) and at the end (day 35) of the BMP test. Different lowercase letters (a, b, c, or d) show significant differences among samples ($p < 0.05$). Values of SE (n = 3) in brackets. TS—total solids; VS—volatile solids.

	BMP Test					
	Day 0			**Day 35**		
Sample	**TS (%)**	**VS (% TS)**	**pH**	**TS (%)**	**VS (% TS)**	**pH**
AS1-0*	$7.91_{(0.11)a}$	$83.87_{(0.20)a}$	$7.21_{(0.02)a}$	$3.59_{(0.06)a}$	$69.95_{(0.08)a}$	$7.27_{(0.05)b}$
AS1-100*	$6.32_{(0.30)b}$	$84.13_{(0.67)a}$	$4.88_{(0.14)c}$	$3.20_{(0.02)a}$	$68.72_{(0.12)b}$	$7.42_{(0.02)a}$
AS1-10*	$6.15_{(0.05)b}$	$83.53_{(0.08)a}$	$5.81_{(0.03)b}$	$3.26_{(0.23)a}$	$69.42_{(0.41)ab}$	$7.38_{(0.02)ab}$
Whey	$7.80_{(0.05)a}$	$78.59_{(0.16)b}$	$4.60_{(0.03)d}$	$1.58_{(0.01)b}$	$61.78_{(0.14)c}$	$7.12_{(0.01)c}$

4. Conclusions

The results demonstrate the effectiveness of cattle slurry acidification using whey, which is a byproduct of the dairy industry. The technique showed the best results in reducing NH_3 emissions, but could also be used as a global mitigation solution to the problem of GHG emissions. A whey dose of 40% (W40) used for cattle slurry acidification showed the best performance, since it was the lowest amount of whey necessary to reach the target pH of 5.5. In relation to slurry storage, the best conditions to minimize GHG and NH_3 emissions were: (1) AS1 scenario that resembles the conditions in typical medium-sized dairy farms from the Italian Alto Adige region, in which slurry is added to the storage tank daily, using a volume that corresponds to 5% of its initial content; (2) using a low organic loading rate of whey during the dynamic storage phase (AS1-10). A drop in the total GHG emissions observed in acidified slurries during the storage phase was mainly due to the lower CH_4 loss followed by CO_2 and N_2O emissions. Furthermore, the batch-type anaerobic co-digestion of acidified and digested slurries resulted in a significant increase in methane production in comparison with the control using non-acidified slurry. Adoption of these results by dairy farms and cheese factories could solve the economic and environmental problems of GHG and NH_3 emissions during manure storage, and the treatment and disposal of whey generated as cheese byproduct, while at the same time increasing the energy and environmental sustainability of these establishments.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The following are supplementary data to this article:

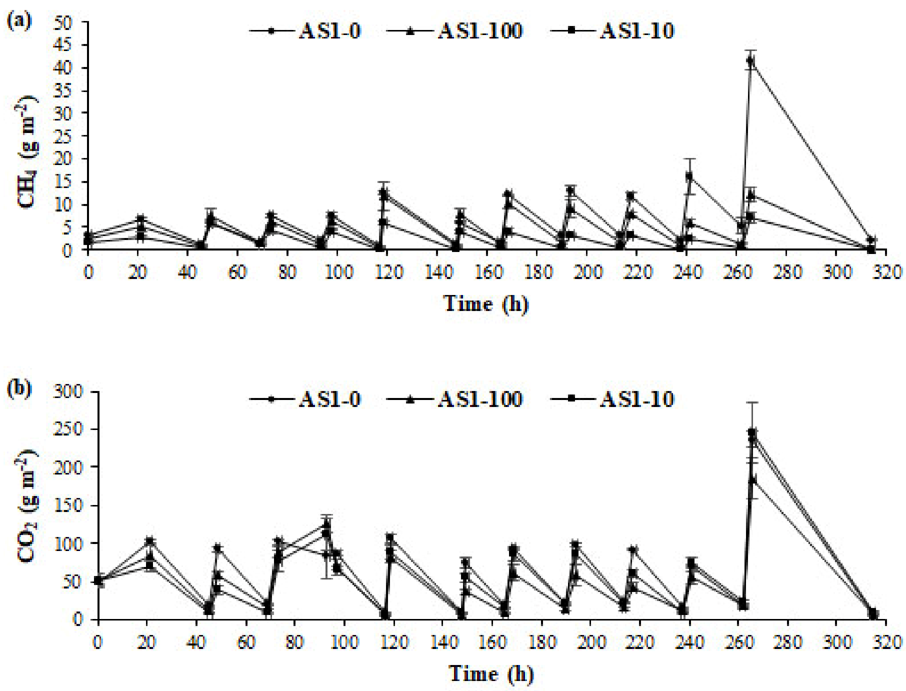

Figure A1. *Cont.*

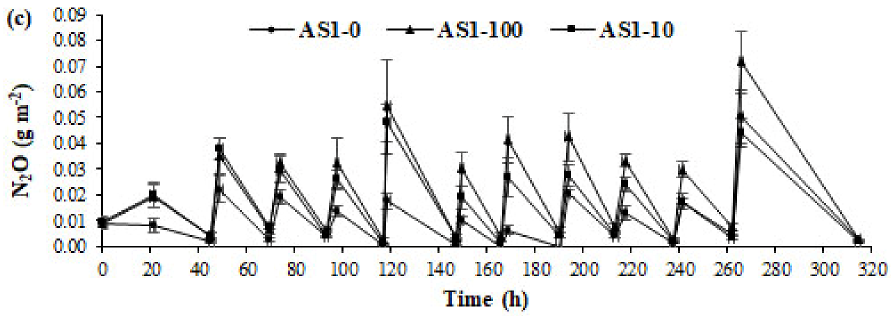

Figure A1. Time course profile of (**a**) CH_4, (**b**) CO_2, and (**c**) N_2O emissions from acidified slurries AS1-10 and AS1-100, and control sample AS1-0. Gases fluxes were measured before and after the daily addition of raw slurry. Error bars indicate the SE (n = 3).

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