

Article

Animal Slurry Sanitization through pH Adjustment: Process Optimization and Impact on Slurry Characteristics

Joana Rodrigues ^{*}, Paula Alvarenga , Ana Carla Silva, Luísa Brito , Jorge Tavares and David Figueiro 

LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; palvarenga@isa.ulisboa.pt (P.A.); acsilva@isa.ulisboa.pt (A.C.S.); lbrito@isa.ulisboa.pt (L.B.); jorgetavares@isa.ulisboa.pt (J.T.); dfangueiro@isa.ulisboa.pt (D.F.)

* Correspondence: joanarodrigues@isa.ulisboa.pt

Abstract: Sanitization by pH adjustment of dairy and pig slurries was evaluated for potential use as organic fertilizer in horticulture. This requires absence of *Salmonella* in 25 g of slurry and less than 1000 *Escherichia coli* colony-forming unit per gram of fresh slurry (Regulation (EU) 2019/1009). Additives used in the alkalization and acidification treatments, included hydroxide-salts and nitrogen-based reactants to increase slurry pH to a basic range (9.0–11.0) and concentrated H₂SO₄ to decrease slurry pH to an acidic range (5.5–3.5). While low-cost urea was unable to increase the slurry pH above 9.5, ammonia efficiently increased slurry pH to the targeted values (but enhanced the emissions risk), whereas the effect of Ca(OH)₂ was hindered by its low solubility. Slurry sanitization by alkalization was achieved at a pH of 9.5 for both slurries, using similar quantities of KOH or NaOH. KOH was selected for further tests since it provides a plant macronutrient. Acidification with concentrated H₂SO₄ was able to achieve sanitization by lowering the pH to 5.0. After a 60-d storage experiment with raw and treated slurries, the level of *E. coli* was below the sanitization limit for all samples. Storage had no significant impact on slurry characteristics, except for ammonium-nitrogen content. Acidification treatment minimized ammonia losses.

Keywords: pig slurry; dairy slurry; acidification; alkalization; *Escherichia coli*; slurry storage



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1. Introduction

The EU-27 has an important population of livestock (143 million pigs, 77 million bovine animals and 74 million sheep and goats [1]), and models predict a continued growth in global demand for livestock products [2]. In some regions, the combined industrialization and geographic concentration of livestock have contributed to the increase of animal slurry (AS) with a deficit of soil area to receive it. Additionally, an incorrect AS management can lead to emissions of ammonia (NH₃) and greenhouse gases (GHG) [3–5], as well as cause: Visual pollution, malodours and contamination of soil and water with pathogens [6]. Consequently, there is an urgent need to develop new management strategies for AS, including the identification of additional soil areas that might receive them and new crops that would benefit from their application.

In many EU countries (e.g., France, Portugal, Spain, and The Netherlands), dairy and pig production co-exists with industrial horticulture that requires important inputs of nutrients. Agricultural valorization of AS as organic fertilizer/soil amendments in food crops, located near a dairy-farm, could be a win-win situation as it would minimize the problems associated with AS management and reduce the use of chemical fertilizers, contributing to a circular economy and a sustainable and integrated production system.

However, the application of raw AS to food crops, namely in horticulture, could be hazardous as AS may contain foodborne pathogens leading to contamination of fresh products [7]. Hence, the use of AS for horticulture requires the absence of *Salmonella* in 25 g of slurry and less than 1000 colony forming units (CFU) of *Escherichia coli* per gram of fresh slurry [8]. In consequence, AS sanitization is mandatory for this purpose. Storage

is the traditional, simplest and cheapest method of AS sanitization before agricultural valorization, since the multiplication of most pathogenic bacteria and parasites is impaired, leading to their attenuation [9,10]. However, the total sanitization of slurry by storage might not be achieved, since in most animal farms, the slurry pit is continuously receiving fresh slurry [10], leading to large variations in effective storage time. Several treatments to obtain a sanitized slurry are available, for instance: Pasteurization, electro-technology, microwaves, pressurization, ultrasounds, and anaerobic digestion [11]. However, some of these treatments imply considerable modifications of the AS management chain, or require a strong investment, being marginally used. The adjustment of the pH of the slurry (by addition of alkali or acids), to values that hinder microbial activity, could be a simple and low-cost solution for AS sanitization. Depending on the additive used, these procedures could improve the AS fertilizer value, and decrease the environmental risk of their application. Slurry acidification with concentrated sulphuric acid (conc. H_2SO_4) is usually practiced in Northern European countries (e.g., Denmark) to reduce harmful NH_3 emissions [12], and previous studies showed that AS acidification affects *E. coli* survival [13,14]. The use of alkali amendments could be another interesting strategy. Chemical treatment with lime products is a simple and cost-effective sanitization process for animal slurry, in which quicklime (calcium oxide $[CaO]$) or hydrated/slack lime (calcium hydroxide $[Ca(OH)_2]$) are added to increase the slurry pH to a high alkaline range ($pH > 12$), effectively reducing the concentration of pathogens. At pH values higher than 12, pathogens cell membranes are destroyed, and high fractions of non-ionised ammonium will act as a biocide [15,16]. Additionally, free calcium ions, present in the lime solution, form complexes with odorous sulphur species, reducing malodours, and metals present in AS precipitate due to the high pH, reducing their solubility, availability, and associated environmental risks [17]. Several studies describe the effectiveness of lime products in reducing microbiological contamination in wastewater [15–19]. However, prior to soil application, the highly alkaline effluent created requires subsequent neutralization with other chemicals. Urea and ammonia were previously indicated as suitable animal waste disinfectants [20,21], despite the concerns about ammonia emissions. A combined treatment could solve this problem with alkalization to achieve sanitization, followed by neutralization to $pH = 7.0$ to reduce emissions [22]. The potential negative impacts on soil properties and availability of nutrients to crops induced by the application of slurries with low or high pH values would also be avoided [23]. These three pH-based approaches to AS sanitization (acidification, alkalization, and combined alkalization/neutralization) need to be thoroughly evaluated and the impact of these treatments on AS characteristics compared. Furthermore, an integrated assessment, considering several additives and the two main animal slurries (dairy and pig), as well as the impact of storage time, also need to be considered to obtain an overall solution.

Therefore, the main goal of this study was to optimize slurry sanitization through pH adjustments. The associated specific objectives were to: (i) optimize different slurry treatments (additives and doses) by alkalization (AL), acidification (AC) and alkalization followed by neutralization (NE); (ii) assess the impact of the treatments on slurries physicochemical characteristics, *E. coli* content and fertilizer value; and (iii) to evaluate the influence of 60-d storage on the composition and sanitization of raw and treated AS.

2. Materials and Methods

2.1. Characterization of Dairy and Pig Slurries

Fresh pig slurry (PS) was collected from an intensive pig breeding farm (Valgado Swine—Olho Cinzeiro) located in Alcochete, Setubal district ($38^{\circ}41'6.5''$ N $8^{\circ}57'44.9''$ W), directly from the discharge pipeline that leads from the animal houses to the slurry reception pit. Dairy slurry (DS) was collected from the main slurry storage tank in a commercial dairy farm (Casa do Gaiato) located in Setubal ($38^{\circ}33'44.7''$ N $8^{\circ}49'38.0''$ W).

Considering the high degradability of AS and to avoid aerobic degradation or fermentation, slurry samples used in all the experiments were collected repeatedly between

February and May 2019. Each sample was identified with a specific code: DS for dairy slurry, PS for pig slurry, and a number identifying the sampling (two for dairy slurries—DS1, DS2 and three for pig slurries—PS1, PS2, PS3). Slurries were characterized for their physicochemical parameters (pH, dry matter (DM) content, organic matter (OM) content, total Kjeldahl nitrogen (TN), ammonium N (N-NH_4^+), total phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), sulphur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B) concentrations) and microbiological composition (detection of *Salmonella* in 25 g of slurry and enumeration of *E. coli* (CFU g^{-1})).

2.2. Physicochemical Analysis of the Slurries

The DM content was determined gravimetrically, at 105 °C, to constant weight, and the OM content by loss on ignition, after calcination at 550 °C for 4 h. A conversion factor of 1.724 is commonly used to convert OM to organic carbon (OC), assuming OM contains 58% OC [24]. The TN was analysed in the fresh samples by the Kjeldahl method, following the digestion, distillation, and titration steps [25], while the N-NH_4^+ content was determined directly, by distillation and titration. Other elements (P, K, Na, Mg, Ca, S, Cu, Zn, Fe, Mn and B) were determined by acid digestion at 105 °C with a 9:3 (*v/v*) mixture of HCl (37% (*w/w*) HCl, $d = 1.19 \text{ kg L}^{-1}$) and HNO_3 (65% (*w/w*) HNO_3 , $d = 1.39 \text{ kg L}^{-1}$), respectively, followed by quantification by inductively coupled plasma—mass spectrometry (ICP-EOS). Samples were analysed in triplicate.

2.3. Microbiological Analysis of the Slurries

2.3.1. Detection of *Salmonella*

The method for the detection of *Salmonella* was based on ISO 6579-1, a horizontal method for the detection of *Salmonella* in animal faeces and environmental samples from the primary production stage [26]. The sample was submitted to a pre-enrichment step, in a non-selective medium (buffered peptone water), incubated at 37 °C for 18 ± 2 h. Afterwards, a selective enrichment was performed onto plates of modified semi-solid Rappaport-Vassiliadis (MSRV, Biokar, Beauvais, France) inoculated with the pre-enriched sample and incubated at 41.5 ± 1 °C for $(2 \times) 24$ h. Plates of xylose lysine desoxycholate (XLD, Biokar, Beauvais, France) were then inoculated from the selective enrichment and incubated during 24 h at 37 °C, for the detection of typical or suspected colonies. The presence or absence of *Salmonella* was reported to 25 g of fresh material. The analyses were replicated using three biologically independent samples (biological replicates), with two repetitions under identical conditions (technical replicates).

2.3.2. Enumeration of *Escherichia coli*

The method for the enumeration of *E. coli* in AS samples was performed in accordance with ISO 16649-2, a horizontal method for the enumeration of beta-glucuronidase-positive *E. coli* by colony-count technique at 44 °C, using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide [27]. As recommended for animal faeces, 1:10 dilutions of the samples in Ringer solution were prepared and kept during 20 h in an orbital shaker at +5 °C, to favour the release of the microorganisms from slurry particles, particularly important in non-homogeneous materials. Afterwards, Tryptone-Bile-Glucuronic plates (TBX, Biokar, Beauvais, France) were inoculated with serial decimal dilutions of the samples. The inoculated plates were incubated for 18–24 h at 44 °C, and the typical β -glucuronidase-positive (blue colour). *E. coli* colony-forming units were counted and the number of CFU of β -glucuronidase-positive *E. coli* per g of slurry was calculated. The analyses were replicated using three biological replicates and two technical replicates.

2.4. Slurry Alkalinization Tests

In the first set of experiments, the performance of different alkalinizing agents was compared, targeting pH values of 9.0, 10.0, and 11.0. Five different additives were tested: hydroxide salts (potassium hydroxide (KOH), sodium hydroxide (NaOH), calcium hydroxide

(Ca(OH)₂), and N-based additives, urea (CH₄N₂O) and ammonia (NH₃). All reactants were used in the solid form, except for ammonia (24% (w/w) NH₃ solution, d = 0.901 kg L⁻¹).

Samples of 500 g of slurry (DS or PS) were alkalized by adding, gradually, small amounts of additives (0.1–1 g), or small volumes of NH₃ (0.1–1 mL), with continuous stirring. The pH value was measured after each addition, using an electrode pH meter. Titration curves were plotted, in a range of pH from 7.0 to 11.5.

Based on the amount of additives needed and on the feasibility of the process, the best additive, KOH, was selected. Once the target pH values were reached (9.0, 9.5, 10.0, and 11.0), the number of *E. coli* (CFU g⁻¹) in the treated slurry samples, was evaluated. All slurry alkalization tests were performed in triplicate.

When slurry pH increases, a shift in the NH₄⁺:NH₃ equilibrium occurs enhancing the risk of NH₃ emissions during storage and after soil application. To mitigate these hazardous emissions and negative impacts on soil quality and productivity, a combined treatment with alkalized/neutralized slurry was also performed. In this test, 500 g of slurry (DS or PS) were alkalized adding enough KOH to reach the pH target value of 9.5. After 24 h, the slurry was neutralized to pH 7.0 by adding conc. H₂SO₄ (95% (w/w) H₂SO₄, d = 1.84 kg L⁻¹).

2.5. Slurry Acidification Tests

Samples of 500 g of slurry (DS or PS) were acidified by using conc. sulphuric acid (95% (w/w) H₂SO₄, d = 1.84 kg L⁻¹), to the target pH values: 3.5; 4.5; 5.0 and 5.5. The acid was gradually added to the slurries in small volumes (1–0.1 mL) with continuous stirring and pH was measured, after each addition. Titration curves were plotted, in a range of pH from 7.5 to 3.5. Once the target pH values were reached (3.5; 4.5; 5.0 and 5.5), the number of *E. coli* (CFU g⁻¹) in the acidified slurry samples were accessed. All slurry acidification tests were performed in triplicate.

2.6. Storage Experiment Setup

Based on the conditions defined in the previous experiments to achieve sanitization of the slurries (acidification to pH 5.0 and alkalization to pH 9.5, using H₂SO₄ and KOH, respectively), a 60-d storage experiment was performed with 5 kg of raw slurries (DS2 and PS3) and with 5 kg of their treated counterparts. For slurry acidification (AC), 45 and 35 mL of H₂SO₄ were added to PS or DS, respectively. Slurry alkalization (AL) to pH 9.5 was achieved by adding 55 g and 40 g of KOH to PS or DS, respectively. For the alkalization/neutralization (NE) treatment, 5 kg of slurry were alkalized to pH 9.5 (as previously described), followed by slurry neutralization to pH 7.0 by H₂SO₄ addition (45 and 35 mL, for PS and DS, respectively). The reactants were mixed with the slurries, in triplicate, 24 h before the beginning of the storage experiment (day 0). Replicates of raw and treated slurries were stored for 60 d in non-hermetically closed plastic containers at ambient temperature. Slurry containers were covered to protect from rainfall, insects and other animals, as well as to reduce odours and ammonia losses. One sample of each replicate (±250 g) was collected at the beginning of the experiment (t = 0 d), and at t = 15 d, t = 30 d and t = 60 d, for subsequent microbiological analysis (except t = 30 d) and physicochemical characterization. Before sampling, the slurry was manually stirred for proper homogenization.

2.7. Statistical Analysis of the Data

To test the effects of the slurry sanitization treatments and storage time, and their interaction (sanitization treatment × storage time) on the microbial and physicochemical characteristics of the slurries, a two-way ANOVA was performed. Before ANOVA, the normality and homogeneity of the variances were checked by the Shapiro Wilk, and Levene test, respectively. Three levels of significance were considered: $p \leq 0.05$, 0.01, and 0.001. Whenever significant differences were found ($p \leq 0.05$), a post hoc Tukey HSD (Honest Significant Difference) test was used to further elucidate differences between

means ($p \leq 0.05$). The statistical software package used was Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Slurry Sanitization by pH Modification

According to the European Regulation (EU) 2019/1009 [1], the legal microbiological criteria for fertilizers is the absence of *Salmonella* in 25 g of material and less than 1000 CFU of *E. coli* per g of fresh material. None of the slurry samples used in this study were contaminated with *Salmonella* but all the samples exceeded the legal limit for *E. coli* (Table 1). Therefore, the sanitization procedures targeted the decrease of *E. coli* in slurries. The results had high standard deviations for the *E. coli* counts, mostly due to the heterogeneity of materials.

Table 1. Physicochemical and microbiological characterization of raw dairy slurry samples (DS1, DS2) and raw pig slurry samples (PS1, PS2, PS3) (Mean, n = 3).

Slurry Sample ID		DS1	DS2	PS1	PS2	PS3	
Sampling Date		12 March 2019	21 May 2019	12 February 2019	13 March 2019	21 May 2019	
Physicochemical parameters	pH	7.49	7.13	7.05	7.21	6.69	
	EC	mS cm ⁻¹	10.17	14.13	24.27	26.30	22.77
	DM	g kg ⁻¹	93.8	97.8	87.9	39.1	124.1
	C		409.1	421.4	457.2	393.1	465.6
	NT		33.7	36.1	68.9	116.8	49.6
	N-NH ₄ ⁺		12.7	16.9	44.9	82.9	29.9
	P		8.7	7.2	15.7	20.6	13.0
	K	g kg ⁻¹ DM	28.5	27.4	33.9	59.4	31.6
	Na		11.0	8.4	13.9	27.8	11.6
	Mg		7.3	5.8	9.2	12.3	10.2
	Ca		18.7	14.0	19.5	25.9	21.0
	S		4.4	3.8	9.0	13.1	7.8
	Cu		43	38	150	181	159
	Zn		267	219	583	548	674
	Fe	mg kg ⁻¹ DM	2332	1779	1258	1228	1912
	Mn		314	220	506	619	545
	B		22	27	39	62	56
Microbiological parameters	<i>E. coli</i>	CFU g ⁻¹	6.1(±0.0) × 10 ³	3.2(±1.7) × 10 ³	3.7(±1.3) × 10 ⁵	1.7(±0.4) × 10 ⁵	5.9(±7.5) × 10 ⁵
	<i>Salmonella</i>	detection in 25 g	Absent				

The target pH defined for slurry acidification (pH 5.5) was based on results reported by Fangueiro et al. [13]. For slurry alkalization, that information was not available, and the pH targets were based on studies focused on sludge sanitization. In fact, the use of metal or ammonium hydroxide salts in the sanitization of urban sewage sludge for agricultural valorization is a common practice [28,29]. Most of these studies proved the efficacy of these additives in reducing the number of pathogens at pH values ranging from 9.0 to 13.0.

3.1.1. Alkalinization

To raise the slurry pH to 9.0, 10.0 and 11.0, five different additives were used which had already proven their efficacy as animal waste disinfectants [20,21]. The selection of the best additive for alkalization was based on the: (i) Amount of additive needed, combined with the feasibility and operational conditions of the process, (ii) costs involved, and (iii) potential agronomic value of the additive and potential environmental impacts.

In terms of process feasibility and operational conditions, NaOH and KOH efficiently increased the pH of both AS to the target values, and the doses (g kg⁻¹) required to attain

the target pH were similar (Table 2). Higher doses were required for PS than for DS, mainly due to the greater buffering capacity of PS, which was observed in the titration curves (Figure 1). The dose of $\text{Ca}(\text{OH})_2$ required to reach pH values of 9.0 and 10.0 was higher than those needed for KOH and NaOH (Table 2). However, the main issue with $\text{Ca}(\text{OH})_2$, compared with NaOH and KOH, was its low solubility, hindering its use in a large scale context. The ammonia solution also increased the pH of the slurries, but increasingly larger volumes were needed to achieve small increases in pH: 4.0; 16.0 and 80.0 mL kg^{-1} for DS, and 6.0; 22.0 and 100.0 mL kg^{-1} for PS, to reach pH values of 9.0; 10.0 and 11.0, respectively (Table 2 and Figure 1D). The use of urea was unsuccessful as the pH never raised above 9.5 (Figure 1E) even with application doses above 20 g kg^{-1} . This may be related to a kinetic limitation caused by low urease activity, needed to convert urea to NH_3 .

Table 2. Doses of potassium hydroxide (KOH), calcium hydroxide ($\text{Ca}(\text{OH})_2$), sodium hydroxide (NaOH), urea ($(\text{NH}_2)_2\text{CO}$) (g kg^{-1}), and aqueous ammonia, NH_3 (mL kg^{-1}) necessary to increase the pH of dairy and pig slurries to the target pH values (9.00; 10.00 and 11.00) (Mean, $n = 3$).

Alkaline Additives		Target pH						
		9.00		10.00		11.00		
		DS	PS	DS	PS	DS	PS	
Hydroxide salts	KOH (P)	pH obtained	9.06	9.00	10.04	10.08	11.11	11.00
		Dose (g kg^{-1})	2.0	4.0	6.0	11.0	12.0	19.0
	$\text{Ca}(\text{OH})_2$ (P)	pH obtained	9.05	9.09	10.13	10.09	11.26	11.07
		Dose (g kg^{-1})	4.0	6.0	8.0	11.0	11.0	16.0
	NaOH (P)	pH obtained	8.98	9.05	9.97	10.02	11.15	11.00
		Dose (g kg^{-1})	2.0	3.0	5.0	7.0	10.0	12.0
N-based compounds	Urea (P)	pH obtained	9.16	8.99			(*)	
		Dose (g kg^{-1})	2.5	5.0				
	NH_3 (S)	pH obtained	9.16	9.16	10.03	10.00	10.99	10.92
		Dose (mL kg^{-1})	4.0	6.0	16.0	22.0	80.0	100.0

Notes: DS = Dairy slurry; PS = Pig slurry; P = Pellets; S = Solution; (*) Target pH values of 10.00 and 11.00 were not reached with the addition of urea.

Based on these results, it was concluded that NaOH and KOH were the most suitable additives, while the others were unable to increase pH to all target values or presented serious operational limitations.

A comparison of the cost of the additives was performed based on the range of prices for large scale acquisition [30]: $\text{Ca}(\text{OH})_2$: 100–125€ ton^{-1} , Urea: 234–401€ ton^{-1} , NaOH: 275–304€ ton^{-1} , NH_3 : 334–417€ ton^{-1} and KOH: 251–471€ ton^{-1} . These prices are merely indicative but provide a clear perspective on differences among additives. The market price for NH_3 solutions is comparatively higher than the prices for hydroxide salts. The alkalization with NH_3 seems thus less attractive, while the use of $\text{Ca}(\text{OH})_2$ would be the cheapest option. Urea was recommended by Ottoson et al. for low level animal waste disinfection due to its low price and large availability [21]. Since there is a price overlap between NaOH and KOH (even if the range of prices for KOH is wider), these two additives are equivalent regarding acquisition costs.

The use of KOH enhances K availability in the soil, a major plant macronutrient, with an important role in the synthesis of organic compounds [23]. In fact, the application of K fertilizers is generally required due to insufficient availability to plants in most soils [23]. The use of NaOH would be a source of Na, a beneficial element for some plant species, and even essential for halophytes [31]. However, Na in soil derives from many sources and its concentration is already higher than the acceptable range in many circumstances [32]. A build-up in soils resulting from further addition of Na may lead to soil deflocculation

(degradation of soil structure) [32]. The use of N-rich additives is a good strategy since it increases the fertilizer value of the AS. Nevertheless, handling and direct addition of aqueous ammonia to AS strongly increases the risk of NH_3 volatilization relatively to the raw slurry. Ottoson et al. proposed low-level treatments with aqueous ammonia for bovine manure disinfection but alerted for the NH_3 emissions and their environmental impact [21].

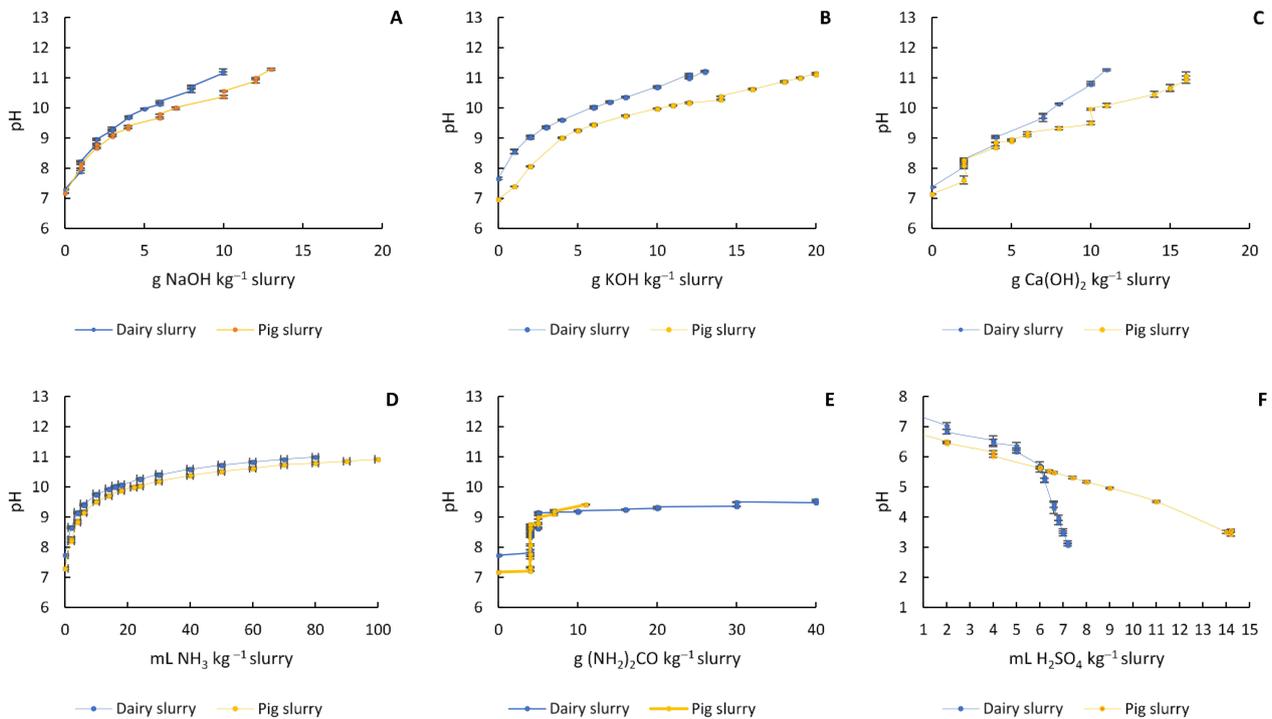


Figure 1. Titration curves for dairy and pig slurries (DS or PS) alkalized with sodium hydroxide (A), potassium hydroxide (B), calcium hydroxide (C), ammonia (D) and urea (E), and titration curves for dairy and pig slurries acidified with sulfuric acid (F).

Another problem of this treatment is the risk of nitrate leaching after AS application to soil, a practice regulated by the Council Directive 91/676/EC of 12 December 1991 concerning water protection against pollution caused by nitrates from agricultural sources [33]. Taking all these facts into consideration, and from an agronomic and environmental perspective, KOH was the best option as a potential alkalizing agent for slurry sanitization and subsequent use as an organic fertilizer in horticulture. It was, thus, selected as the alkalizing agent for a new set of PS and DS treatments (Table 3). In this part of the study, the *E. coli* counts in the treated slurry samples showed that the legal criterion for sanitization ($<1000 E. coli$ CFU g^{-1}) was achieved in both AS samples when the pH of the slurry was increased to 9.5 with KOH (Table 3). Furthermore, the *E. coli* counts of the treated samples, relatively to their raw counterparts (Table 1), was reduced to values below the detection limit of the enumeration method ($<100 E. coli$ CFU g^{-1}) using a minimum dose of KOH of 5.0 g kg^{-1} for DS and 7.0 g kg^{-1} for PS (Table 3).

Table 3. Doses (g kg^{-1} slurry) of potassium hydroxide (KOH) used to increase the pH of dairy and pig slurries to the target pH values (9.00; 9.50; 10.00; and 11.00), and *E. coli* (CFU g^{-1}), before and after alkalization of the slurry samples; and doses (mL kg^{-1} slurry) of concentrated sulphuric acid (H_2SO_4) used to decrease the pH of dairy and pig slurries to the target pH values (5.50; 5.00; 4.50 and 3.50), and *E. coli* (CFU g^{-1}) before and after acidification of the slurry samples (Mean, $n = 3$).

pH target		9.00		9.50		10.00		11.00	
AL	AS sample ID	DS1	PS1	DS1	PS2	DS1	PS1	DS1	PS1
	KOH (g kg^{-1})	3.0	4.0	5.0	7.0	8.0	12.0	14.0	21.0
	pH reached	9.01	9.01	9.53	9.50	10.02	10.00	11.01	11.09
	<i>E. coli</i> (CFU g^{-1})	$4.50(\pm 0.71) \times 10^2$	$2.90(\pm 1.60) \times 10^5$	<100	<100	<100	<100	$0.50(\pm 0.71) \times 10^2$	n.a.
pH target		5.50		5.00		4.50		3.50	
AC	AS sample ID	DS1	PS2	DS1	PS2	DS1	PS1	DS1	PS1
	H_2SO_4 (mL kg^{-1})	6.00	6.40	6.20	8.00	6.60	9.08	7.00	14.00
	pH reached	5.60	5.51	5.33	5.15	4.44	4.95	3.45	3.51
	<i>E. coli</i> (CFU g^{-1})	$1.00(\pm 1.00) \times 10^2$	$2.39(\pm 2.00) \times 10^4$	$1.50(\pm 1.97) \times 10^2$	$1.50(\pm 3.68) \times 10^2$	$1.00(\pm 0.63) \times 10^2$	<100	<100	<100

Note: AS = animal slurry; ID = sample identification; DS = Dairy slurry; PS = Pig slurry; AC = Acidified slurry; AL = Alcalinized slurry; n.a. = not analysed.

3.1.2. Acidification

Slurry acidification by addition of concentrated H_2SO_4 is a usual practice in Northern European countries (e.g., Denmark), recommended by the authorities to reduce harmful NH_3 emissions [12]. Nevertheless, the impact of slurry acidification on microbial activity has been marginally considered and results are contradictory. Indeed, Fangueiro et al. reported that AS acidification to pH 5.5 increased the *E. coli* population and the risk of its leaching after application of the acidified slurry to soils [13]. Soares et al. also indicated a temporary increase in the *E. coli* population after acidification to pH 5.5 [14]. A pH value of 5.5 was set as the target value in acidification studies to prevent NH_3 emissions during storage [12]. However, this pH value increased over time, requiring an extra acid addition [34]. Regueiro et al. reported that slurry acidified to pH 3.5 maintained this pH value for a longer period during storage. Hence, three pH target values were considered in the present study: 5.5, 4.5 and 3.5 [34].

While for DS the number of CFU obtained after acidifying to pH 5.5 was $1.00 (\pm 0.63) \times 10^2$, below the legal limit required, that pH adjustment was not enough to achieve sanitization of PS as the number of *E. coli* CFU was still higher than the legal limit (Table 3). At pH 5.0, the numbers in both slurries decreased to $1.50 \times 10^2 \text{ CFU g}^{-1}$, below the legal limit, and at pH values of 4.5 and 3.5 the number of CFU were even below the detection limit of the method ($<100 \text{ CFU g}^{-1}$, Table 3).

Therefore, AS acidification with H_2SO_4 to pH 5.0 (with 9 mL $\text{H}_2\text{SO}_4 \text{ kg}^{-1}$ PS and 6.4 mL $\text{H}_2\text{SO}_4 \text{ kg}^{-1}$ DS) was enough to achieve legally admissible concentrations of *E. coli*. The amount of H_2SO_4 used to sanitize the slurry did not exceed 1.5% (w/w), which represents an affordable treatment if implemented in field conditions [34].

Animal slurry acidification prevents significant mineral N losses since at $\text{pH} < 5.5$, 99% of ammonia remains as ammonium [12]. Furthermore, several studies indicated that repeated applications of acidified slurry had a low impact on soil pH and no impact on soil microbial communities [35–37].

However, concerns remain about the risks associated with H_2SO_4 handling, requiring trained staff, due to its (i) corrosiveness, (ii) possible development of volatile sulphur-containing compounds (H_2S) and (iii) foam formation during the addition to the slurry,

limiting its wider use [12]. As alternatives for slurry acidification, other acids were studied by Regueiro et al. and Kavanagh et al. [34,38], but none were so efficient and cost-effective as H₂SO₄. Furthermore, H₂SO₄ supplies sulphur, an important plant macronutrient [34].

The doses identified in the present study to achieve sanitization by alkalization or acidification should be taken as indicative only, as the initial pH and total solids have a paramount influence on the doses needed to achieve the targeted pH values.

3.1.3. Slurry Characteristics That Affect pH Adjustment and Sanitization

The results showed that the physicochemical characteristics of the raw AS (Table 1) influenced the alkali/acid doses needed to achieve target pH values (Tables 2 and 3, and Figure 1). Generally, the amounts of additives required were greater for PS than DS. Indeed, despite the similar pH and C content of both slurries, major differences were found in nutrient content (e.g., N-total, P, K, Mg, Ca, S, Cu, Zn), higher in PS (Table 1), which may explain the higher doses needed to achieve the same pH in this slurry.

It should be noted that the DM content was higher in raw DS than in raw PS (Table 1). Previous studies reported that the DM content of the slurry has a strong influence on the amount of additive needed to modify the pH value [34,39], but such effect was not observed in the present study. Nevertheless, higher DM contents hindered the homogenization of the mixture of slurry and additives and might have an impact on the overall sanitization process, namely on the time needed to achieve the equilibrium pH.

Generally, DS particles were larger than PS particles [40]. Larger particles may have reduced the treatment efficiency and protected pathogens in the slurries from chemical stress. Furthermore, it also caused difficulties in sampling and analysis.

3.2. Influence of Storage Time on the Sanitization of Raw and Treated AS

Microbial survival during slurry storage depends on (i) slurry source, (ii) level of contamination, (iii) nutrients availability, (iv) dry matter content, (v) redox potential, (vi) type of microbiota and (vii) temperature [41]. Minimum storage time for the safe management of raw slurry in agriculture may differ between regions with different climate types. Generally, a 4-month storage period is enough, but in regions with cold climates (e.g., Nordic countries), it may be necessary to prolong the storage period for up to one year [10,42].

As mentioned in Section 3.1, even before sanitization and storage there was no evidence for the presence of *Salmonella* in the AS samples used, but the levels of *E. coli* were 3.22×10^3 and 5.96×10^5 CFU g⁻¹ in DS and PS samples used in the storage experiment, respectively (Table 4).

Table 4. *E. coli* (CFU g⁻¹) in raw and treated dairy and pig slurry samples (DS and PS), at the beginning of the storage test (t = 0 d) and after 15 and 60 days (t = 15 d; t = 60 d) (Mean, n = 3). Slurry samples were acidified to pH 5.0 (AC), alkalized to pH 9.5 (AL), and a combined treatment alkalization/neutralization (NE) was also tested.

Slurry Type	Treatment	<i>Escherichia coli</i> (CFU g ⁻¹)		
		Storage Time		
		t = 0 d	t = 15 d	t = 60 d
DS (sample DS2)	RS	3.22(±1.69) × 10 ³	1.57(±2.54) × 10 ³	<100
	AC	<100	-	<100
	AL	<100	<100	<100
	NE	<100	<100	<100
PS (sample PS3)	RS	5.96(±7.49) × 10 ⁵	2.33(±1.53) × 10 ²	<100
	AC	<1000	<100	<100
	AL	<100	<100	<100
	NE	<100	<100	<100

Note: CFU = Colony-forming unit; DS = Dairy slurry; PS = Pig slurry; RS = Raw slurry; AC = Acidified slurry; AL = Alkalized slurry; NE = Neutralized slurry.

After 15 d of storage of raw slurries, a decrease in the number of *E. coli* CFU g⁻¹ was observed (Table 4) in DS (1.57 (±2.54) × 10³) and PS (5.30 (±3.34) × 10³), but the counts remained above the legal limit. However, after 60 d of storage, the levels of *E. coli* were <100 CFU g⁻¹, indicating a complete sanitization of both raw DS and PS samples (Table 4). This result is in agreement with Heinonen-Tanski et al., who reported that storage for long periods before soil application is enough to ensure sanitization, since pathogen survival in manure lasted only for few days at high temperatures (20–40 °C) and few weeks at low temperatures (4–10 °C) [9]. Similarly, Wang et al. reported that *E. coli* in bovine faeces survived for less than 50 d at 37 °C and for 49 to 56 d at 22 °C [43].

The sanitization treatments by pH increase to 9.5, combined treatment (alkalinization followed by neutralization) and acidification to pH = 5.0, in both DS and PS, kept the *E. coli* counts below the legal limit (<1000 CFU g⁻¹) after the 60 d of storage (Table 4).

3.3. Influence of the Treatment and Storage Time on the Physicochemical Characteristics of Raw and Treated AS

The PS was richer than the DS in most nutrients and OM content. The treatments and the storage influenced their physicochemical characteristics, which were significantly different at the end of the storage period (t = 60 d), both in the raw slurries and in their treated counterparts ($p < 0.05$, 0.01 or 0.001, depending on the parameter) (Tables 5 and 6).

Table 5. Physicochemical analysis (pH, EC, DM, OM, TN, NH₄⁺-N, P and K) of raw and treated dairy slurry (DS2), at the beginning of the storage test (t = 0 d) and after 15, 30 and 60 days (t = 15, t = 30 and t = 60 d) (Mean, n = 3). Slurry samples were acidified to pH 5.0 (AC), alkalized to pH 9.5 (AL), and a combined treatment alkalization/neutralization (NE) was also tested.

DS	Time (Days)	pH	EC (mS cm ⁻¹)	DM (g kg ⁻¹)	g kg ⁻¹ (DM)				
					C	TN	NH ₄ ⁺ -N	P	K
	0	7.13 ^h	14.13 ^g	97.8 ^k	421.4 ^a	36.1 ^a	16.9 ^a	7.2 ^{c,d}	27.4 ^{e,f}
RS	15	7.19 ^h	12.83 ^{g,h}	123.5 ⁱ	396.6 ^{b,c}	30.4 ^d	11.5 ^d	8.1 ^{a,b,c}	26.4 ^f
	30	7.75 ^f	11.85 ^{h,i}	161.1 ^f	383.0 ^d	29.8 ^d	8.3 ^f	8.3 ^{a,b}	29.9 ^e
	60	8.95 ^c	11.38 ⁱ	218.0 ^c	371.8 ^e	22.4 ^g	3.9 ⁱ	8.8 ^a	27.9 ^{e,f}

Table 5. Cont.

DS	Time (Days)	pH	EC (mS cm ⁻¹)	DM (g kg ⁻¹)	g kg ⁻¹ (DM)				
					C	TN	NH ₄ ⁺ -N	P	K
AC	0	5.05 ^l	18.13 ^{e,f}	108.2 ^j	405.4 ^b	33.8 ^{b,c}	15.3 ^{b,c}	6.6 ^{d,e}	23.8 ^g
	15	5.28 ^k	17.53 ^f	146.8 ^g	396.52 ^c	33.9 ^b	15.2 ^c	6.7 ^{d,e}	22.4 ^g
	30	5.70 ^j	20.43 ^{b,c}	191.0 ^d	395.13 ^c	33.6 ^{b,c}	15.0 ^c	7.1 ^{d,e}	23.5 ^g
	60	6.76 ⁱ	21.90 ^a	276.5 ^a	393.15 ^c	32.5 ^{b,c}	15.1 ^c	6.1 ^e	23.2 ^g
AL	0	9.23 ^a	19.47 ^{c,d}	101.7 ^{i,k}	393.4 ^c	32.8 ^{b,c}	15.7 ^b	6.1 ^e	59.9 ^{b,c}
	15	8.28 ^d	18.23 ^{d,e,f}	133.6 ^h	372.4 ^e	27.2 ^e	8.7 ^f	6.9 ^{d,e}	59.7 ^c
	30	8.35 ^d	19.20 ^{c,d,e}	160.6 ^f	371.7 ^e	24.3 ^f	5.9 ^h	7.2 ^{c,d}	67.0 ^a
	60	9.27 ^a	20.20 ^{b,c}	215.7 ^c	362.5 ^f	20.3 ^h	3.4 ^j	7.3 ^{b,c,d}	62.2 ^b
NE	0	7.20 ^h	21.23 ^{a,b}	107.2 ^{i,k}	372.9 ^e	32.3 ^c	15.1 ^c	6.5 ^{d,e}	60.6 ^{b,c}
	15	7.47 ^g	18.80 ^{d,e,f}	137.2 ^{g,h}	355.6 ^{f,g}	27.0 ^e	9.8 ^e	6.5 ^{d,e}	56.9 ^d
	30	8.04 ^e	21.77 ^a	178.2 ^e	349.2 ^g	23.8 ^{f,g}	6.5 ^g	7.5 ^{b,c,d}	68.0 ^a
	60	9.08 ^b	22.29 ^a	257.3 ^b	348 ^g	18.7 ⁱ	3.3 ^j	7.6 ^{b,c,d}	61.8 ^{b,c}
Two-way ANOVA									
Slurry treatment (T)		***	***	***	***	***	***	***	***
Storage time (t)		***	***	***	***	***	***	***	***
T X t		***	***	***	***	***	***	***	***

DS = Dairy slurry; RS = Raw slurry; AC = Acidified slurry; AL = Alkalized slurry; NE = Neutralized slurry; EC = Electrical conductivity; DM = Dry matter; TN = Total (Kjeldahl) N. *** = significant at $p \leq 0.001$, respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test ($p < 0.05$). n.s. = not significant.

Table 6. Physicochemical analysis (pH, EC, DM, OM, TN, NH₄⁺-N, P e K) of raw and treated pig slurry (PS3), in the beginning of the storage test (t = 0 d) and after 30 and 60 days (t = 15, t = 30, t = 60 d) (Mean, n = 3). Slurry samples were acidified to pH 5.0 (AC), alkalized to pH 9.5 (AL), and a combined treatment alkalization/neutralization (NE) was also tested.

PS	Time (Days)	pH	EC (mS cm ⁻¹)	DM (g kg ⁻¹)	g kg ⁻¹ (DM)				
					C	TN	NH ₄ ⁺ -N	P	K
RS	0	6.69 ⁱ	22.77 ^{e,f}	124.1 ⁱ	465.5 ^a	49.6 ^{b,c}	29.9 ^{a,b}	13.0 ^{a,b,c,d,e}	31.6 ^f
	15	7.14 ^{f,g}	24.07 ^e	141.6 ^h	454.7 ^b	48.3 ^{c,d,e}	26.9 ^e	12.6 ^{b,c,d,e}	36.8 ^e
	30	7.18 ^f	21.90 ^f	168.9 ^{f,g}	451.2 ^b	45.1 ^{f,g}	22.1 ^g	14.1 ^{a,b}	39.6 ^e
	60	7.56 ^e	21.91 ^f	202.3 ^e	432.8 ^e	42.8 ^{g,h}	16.3 ⁱ	14.6 ^a	33.0 ^f
AC	0	5.14 ^l	26.63 ^d	126.2 ⁱ	441.6 ^{cd}	49.1 ^{c,d}	29.1 ^{c,d}	11.7 ^{d,e}	31.8 ^f
	15	5.46 ^k	30.97 ^c	164.2 ^g	443.0 ^c	50.9 ^{a,b,c}	29.5 ^{b,c}	12.3 ^{c,d,e}	30.6 ^f
	30	5.37 ^k	31.37 ^c	196.8 ^e	437.4 ^{de}	52.0 ^{a,b}	30.1 ^{a,b}	12.9 ^{a,b,c,d,e}	30.9 ^f
	60	6.10 ^j	33.62 ^b	293.2 ^b	425.9 ^f	52.4 ^a	30.6 ^a	13.0 ^{a,b,c,d,e}	31.1 ^f

Table 6. Cont.

PS	Time (Days)	pH	EC (mS cm ⁻¹)	DM (g kg ⁻¹)	g kg ⁻¹ (DM)				
					C	TN	NH ₄ ⁺ -N	P	K
AL	0	9.41 ^a	26.83 ^d	125.8 ⁱ	422.1 ^{f,g}	46.2 ^{e,f}	28.3 ^d	12.3 ^{c,d,e}	74.3 ^{b,c}
	15	7.98 ^c	30.60 ^c	162.0 ^g	417.6 ^{g,h}	38.4 ⁱ	21.3 ^g	11.6 ^e	72.9 ^{c,d}
	30	7.73 ^d	26.53 ^d	200.8 ^e	416.4 ^h	33.2 ^j	15.7 ⁱ	13.6 ^{a,b,c}	80.0 ^a
	60	8.35 ^b	26.49 ^d	261.2 ^c	394.9 ^k	30.9 ^j	11.4 ^j	14.2 ^{a,b}	75.7 ^{b,c}
NE	0	7.09 ^{f,g,h}	30.40 ^c	126.7 ⁱ	410.3 ⁱ	46.6 ^{d,e,f}	28.6 ^d	12.4 ^{c,d,e}	74.2 ^{b,c}
	15	7.05 ^{g,h}	33.93 ^{a,b}	171.6 ^f	409.9 ⁱ	42.2 ^h	25.1 ^f	12.7 ^{b,c,d,e}	70.2 ^d
	30	6.98 ^h	32.20 ^{b,c}	215.2 ^d	407.8 ^{i,j}	40.7 ^{i,h}	19.4 ^h	13.8 ^{a,b,c}	77.4 ^{a,b}
	60	8.31 ^b	35.89 ^a	312.7 ^a	403.0 ^j	32.4 ^j	11.6 ^j	13.5 ^{a,b,c,d}	73.9 ^{b,c}
Two-way ANOVA									
Slurry treatment (T)		***	***	***	***	***	***	***	***
Storage time (t)		***	***	***	***	***	***	***	***
T X t		***	***	***	***	***	***	n.s.	***

PS = Pig slurry; RS = Raw slurry; AC = Acidified slurry; AL = Alkalized slurry; NE = Neutralized slurry; EC = Electrical conductivity; DM = Dry matter; TN = Total (Kjeldahl) N. *** = significant at $p \leq 0.001$, respectively. For each parameter, values followed by the same letter do not differ significantly according to the Tukey test ($p < 0.05$). n.s. = not significant.

The DM content of the slurries (RA, AC, AL and NE, for both PS and DS) increased continuously along the 60 d of storage due to a concentration effect caused by water evaporation. At the end of the experiment, raw DS presented a lower DM than the corresponding acidified and neutralized slurries. Previous studies have also shown an increase in DM after the addition of H₂SO₄ [34,44–46], and some authors have attributed the cause to the increase of sulphate concentration [44,45].

The raw AS slurries used in this work had an initial pH value close to 7.0, which increased until the end of the experiment (Tables 5 and 6). At the beginning of storage (one day after treatment), the acidified slurries presented pH values of 5.05 and 5.14 for DS and PS, respectively, while the alkalized slurries had pH values of 9.23 and 9.41 for DS and PS, respectively. The storage time (t), the slurry treatment (T), as well as the interaction between the time and treatment ($t \times T$) had significant effects on slurry pH ($p < 0.01$), for both DS and PS. The pH values of DS and PS acidified to pH 5.0 increased, during the 60 d of storage, to final values of 6.76 and 6.10, respectively. A similar shift was observed, after the same period, in DS and PS samples neutralized to 7.0 with pH increases to 9.08 and 8.31, respectively. In both cases, the pH tended to increase more in the DS than in the PS (Tables 5 and 6), due to the higher buffering capacity of the latter. Eriksen et al. and Regueiro et al. also observed this trend in the pH of acidified slurries and attributed it to microbial activity [34,44]. The gradual pH increase after acidification might also be due to the degradation of dissociated organic acids, as well as to the mineralization of organic N and dissolution of carbonates [46,47].

After 30 d of storage, the pH of the alkalized DS and PS (pH 9.5) decreased to 8.35, and 7.73, respectively. However, by the end of the experiment, pH values had increased again, to 9.27 and 8.35, respectively. This can be explained by the loss of water and the consequent increase in DM observed during storage (Tables 5 and 6). Despite these drifts in pH values of the treated AS over the 60 d storage period, the slurries remained sanitized (Table 4).

The treatments by pH adjustment had a strong impact on the EC of both slurries (Tables 5 and 6), with the higher effect observed for the NE treatment (an increase of 50%

and 35% in EC values of DS, and PS, respectively). Considering that the main objective of the slurry treatment would be its application as an organic fertilizer in horticulture, this increase of EC could lead to problems of salinity during plant growth [48] and impair the AS value as a fertilizer, an issue that needs proper evaluation.

During storage, a two-fold increase in N-NH_4^+ concentration was consistently observed in PS compared to DS (Tables 5 and 6). One important observation was that sanitization by acidification was the only treatment that minimized decreases in N-NH_4^+ concentration associated to NH_3 volatilization, corroborating previous studies that have proposed acidification as a strategy to mitigate NH_3 emissions during storage of AS [49]. In fact, the N-NH_4^+ concentrations in acidified DS and PS were significantly higher ($p < 0.001$) than in all other treatments and remained constant during the whole storage period. In contrast, in the raw, alkalized, or neutralized AS, the N-NH_4^+ concentrations decreased during storage, suggesting loss by NH_3 emission. During the 60-d storage, the total NH_3 losses were similar in neutralized, alkalized and raw DS. In contrast, total NH_3 losses were significantly higher in neutralized and alkalized PS than in raw PS. Moreover, the neutralization approach was not able to reduce NH_3 emissions of AS during storage. A solution to overcome this problem would be the use of biofilters with a high removal capacity to trap ammonia and greenhouse gases (GHG) released during the slurry alkalization process and subsequent storage [50,51].

The TN content decreased in the raw, alkaline, and neutralized AS samples in parallel with the ammonia emissions during the 60 d of storage. The acidified slurry samples showed the highest concentrations of TN, which is in agreement with other studies stating that acidification can increase the AS fertiliser value by preserving N.

The total concentrations of plant macronutrients (Ca and Mg) and micronutrients (Cu, Zn, Fe, Mn, and B), which were initially higher in PS than in DS (except for Fe), were not affected by the sanitization treatments (Tables S1 and S2). The same was true for P total concentration, one of the most important parameters concerning the AS fertilizer value, which remained constant during the whole storage experiment.

As expected, AS sanitization treatments that included KOH addition (AL and NE) led to significant increases in the K total concentration, while the treatments with the addition of concentrated H_2SO_4 (AC and NE) led to significant increases in S total concentration. However, acidified PS and DS lost 27 and 30% of sulphur content, respectively, after 60 days of storage, which may be explained by the activity of sulphate-reducing bacteria, that produce hydrogen sulfide (H_2S). A similar loss in S content was observed in a storage study with digested pig slurry, using mechanically ventilated reactors, performed by Wang et al., in which acidified slurry at pH 5.5 increased H_2S emissions by 11% [52].

3.4. Practical Applications

The proposed slurry sanitization treatments by pH adjustment should be performed by specialized and trained staff. However, they might be easily applied at farm scale if proper training is provided to farmers. Overall, the estimated associated costs of the suggested sanitization treatments, per ton of treated AS (AL to pH 9.5 with KOH: 1.3–2.3 € ton^{-1} DS and 1.8–3.3 € ton^{-1} PS; AC to pH 5.0 with H_2SO_4 : 1.0–3.9 € ton^{-1} DS and 1.4–5.5 € ton^{-1} PS [30]) are similar to standard slurry storage costs (2.3 € ton^{-1} slurry [53]), opening new opportunities for farmers to export slurry to other crops.

4. Conclusions

The present study showed that slurry sanitization by pH adjustment can be a simple and low-cost solution that improves the fertilizer value of AS without generating any new residues.

Sanitization by alkalization was achieved for PS and DS at a pH of 9.5 using similar doses of KOH or NaOH. The additive selected was KOH, as it provides the macronutrient (K) and avoids the accumulation of Na in soils. The remaining additives tested in this study, $\text{Ca}(\text{OH})_2$, urea ($\text{CH}_4\text{N}_2\text{O}$) and ammonia (NH_3), had operative constraints.

The storage for 60 d ensured sanitization of raw AS, but it would require an extended period of time between slurry production and application, with correspondent storage costs, and concomitant risks of NH₃ emissions.

The addition of KOH to increase the pH of the slurry to 9.5, while guarantying sanitization for 60 d of storage, led to NH₃ volatilization during treatment and storage, associated with a decrease in N-NH₄⁺ concentration. Nevertheless, a similar decrease of N-NH₄⁺ concentration was observed in the raw slurries, indicating that alkalization did not increase NH₃ losses relatively to raw slurry.

To mitigate these hazardous emissions, a combined treatment, alkalization/neutralization was also tested. This treatment was also able to ensure AS sanitization throughout storage, but it failed to prevent NH₃ emissions. As an alternative to this combined treatment, another possibility under study, within our group, is the use of biofilters to trap ammonia and greenhouse gases (GHG) released after slurry alkalization and during storage [51].

Animal slurry acidification to pH 5.0 with conc. H₂SO₄ was able to sanitize both PS and DS. This effect remained during the 60 d storage period. Moreover, acidification was the only treatment that minimized NH₃ volatilization during slurry storage. Future work should focus on the dynamics of *E. coli* populations during storage of acidified slurry, in order to validate the efficiency of this treatment. In addition, more information is needed on the fertilizer value of the treated slurries.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/3/517/s1>, Table S1: Physicochemical analysis (Na, Mg, Ca, S, Cu, Zn, Fe, Mn and B) of raw and treated dairy slurry (DS2), in the beginning of the storage test (t = 0 d) and after 15, 30 and 60 days (t = 15, t = 30 and t = 60 d) (Mean, n = 3). Slurry samples were acidified to pH 5.0 (AC), alkalized to pH 9.5 (AL), and a combined treatment alkalization/neutralization (NE) was also tested, Table S2: Physicochemical analysis (Na, Mg, Ca, S, Cu, Zn, Fe, Mn and B) of raw and treated pig slurry (PS3), in the beginning of the storage test (t = 0d) and after 15, 30 and 60 days (t = 15, t = 30, t = 60 d) (Mean, n = 3). Slurry samples were acidified to pH 5.0 (AC), alkalized to pH 9.5 (AL), and a combined treatment alkalization/neutralization (NE) was also tested.

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