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Manure storage and its effect over N-related compounds emissions

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“Life is the art of drawing sufficient conclusions from insufficient premises.”

Samuel Butler

REPORT

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SUMMARY

Agricultural sector is particularly important in Spanish economy, being one of the main European distributors of pork, with a consumption of 1 kg-pork/week per inhabitant (Faostat 2007). However, it has some negative environmental impacts that clearly affect the global warming. According to data available from 2006 in the National Greenhouse Gas Emissions Inventory, GHG emissions from pig sector in Spain amount to 8.8 million *tonnes* of equivalent CO_2 per year. This has led to several regulations which demand the treatment of surplus. Some mitigation strategies might be implemented at farm-level. Consequently, the main objective of this work was to evaluate two mitigation strategies during storage: acid addition and methanogenic activity inhibitors. To that purpose, a state of the art was carried out to establish the different storage strategies and to select the suitable additive. Manure was characterized and an experimental set-up was performed to simulate anaerobic storage conditions. NH_3 and GHG emissions were calculated according to the IPPC Directive tables and a comparison regarding tabulated values of IPPC (European Directive), of attained NH_3 and GHGs emissions with these strategies was made. As a secondary objective, an open chamber was designed and set-up to measure NH_3 and GHG emissions. Therefore, a decrease of pH to 5.5 H_2SO_4 contributed to reduce CH_4 emissions in a 60% and completely inhibit NH_3 but hardly increased SO_4^{2-} concentration to dangerous levels for planted soils. Thus, organic acids were suitable being less harmful and most effective than strong in reduce GHG emissions, in the case of cow slurry mixture, particularly acetic acid. Related to pig manure, lactic acid showed the same mitigation effect as H_2SO_4. RY modulate CO_2 and NH_3 production in the case of the acidification strategy.

Keywords: Ammonia, greenhouse gases, storage conditions, additive, emissions, characterization.

1. INTRODUCTION

Farms modernization is increasing the agrarian activity. Although being associated with a good use of resources it entails environmental risks. The contribution to global warming through GHG emissions (IPC, 2007) is one of the most important ones. Hence, in Spain and especially pig sector, it is established as the most important source of N-related compounds emissions by the Ministry of Livestock, Agriculture and Fisheries. Approximately 40% of the global anthropogenic emissions of NH_3 and N_2O are associated with livestock manure (Galloway et al., 2004; Oenema et al., 2005) and more than 30% of the generated manure is stored as surplus (ADAP, 2013).In this sense, water pollution due to nitrates and air quality alteration has in many cases an agricultural origin, leading the various authorities to establish an increasingly restrictive legislation (Annex 1). All this, leads to the need to study different mitigation measures to reduce these emissions or at least their intensity. This would improve not only the environmental quality but also producer's incomes.

1.1. ENVIRONMENTAL CONCERNS OF MANURE MANAGEMENT

During manure management, important factors are involved leading to significant environmental impacts and entailing health risk for both humans and animals (Table 1).

TABLE 1 EFFECT OF MANURE EMISSIONS ON THE ENVIRONMENT

ENVIRONMENTAL FACTOR	EFFECT ON ENVIRONMENT
Nitrates and phosphates	Water pollution
Phosphorus (P) ingested with food and excreted	Soil and aquifers pollution by accumulation (Zhang H. et al., 2012)
Heavy metals in high concentrations (Cu, Zn, etc.)	Toxicity risks for plants and microorganisms, soil pollution.
Volatile fatty acids (VFA)	Bad odours
Ammonia (NH_3)	Acidification, eutrophication ,volatilization
GHG (CO_2, N_2O and CH_4)	Global warming, ozone layer destruction (IPPC, 2007)

1.2. MANURE AS FERTILIZER

In view to obtain a high yield during the application as organic fertilizer, manure must be as fresh as possible. Royal Decree 824/2005, of 8th July, about fertilizing products, establishes the nutrient content limits to ensure the quality taking into account C/N ratio, P_2O_5 and K_2O content. This depends on the management system (outdoor or indoor raft), storage capacity and fertilizer requirements (Campos-Pozuelo, et al., 2001). During storage, manure properties which later determined it as a good fertilizer are loss. In such way, according to EUROSTAT (2013), in Spain the storage capacity is three months.

1.3. STRATEGIES TO REDUCE EMISSIONS

Animal excreta could be classified according to the type of organic waste generated. Burton (1997) differentiates three groups: (1) solid or semisolid soils, composed by a mixture of animal excreta, straws or sawdust; (2) liquid manure (urine); and (3) wastewater. Regardless of their origin, manure does not contain nitrates at the source. Main factors affecting nitrogen balance are room temperature, manure pH, NH_3 content and air contact surface (Table 2).

TABLE 2 CHARACTERISTICS OF MANURE ACCORDING TO THE PH

pH	Dry matter (g/kg)	S total (g/kg)	N total (g/kg)	NH4-N (g/kg)	VS (g/kg)	Reference
7.0 Untreated	91.0	0.058	7.5	5.5	7.1	Cocolo et al. (2016)
5.3	83.0	4.5	6.4	4.1	6.3	Cocolo et al. (2016)
7.03 Untreated	47.0	0.49	2.65	0.56	39.3	Sorensen et al.(2009)
6.01	50.3	2.04	2.58	0,59	41.3	Sorensen et al. (2009)

Therefore, the wide variety methods to control and delay NH_3 emissions are classified as BAT in: Nitrogen management, Livestock feeding strategies to reduce nitrogen evacuation, accommodation emissions systems reduction, Mitigation measures during storage, as well as acidifying the mix.

Despite the efficiencies obtained by the dietary method for mitigating NH_3 emissions (Dourmand, 1991; Castañeda et al., 1995), many studies proved that NH_3 cannot be reduced until 100% (Osorio et al., 2013; Murphy et al. 2010). Therefore, other operations applicable to the treatment of livestock residues have been studied as the incorporation of additives (Flotats et al. 2000). It should be noted that a lower pH reduces NH_3 content, depending on the equilibrium NH_4^+/NH_3, and affects its bioconversion which includes the formation of NO_x which has not been studied in depth (Liu et al., 2015; Hou Y., 2015).

1.3.1. Selection of the suitable additive for acidification

NH_3 emissions have the drawback that the fertilizer effect of livestock manure is reduced. It is possible to avoid this problem by acidifying the manure to a pH level between 5 and 6 in lack of air (Hyldgaard, 2014).

Short chain organic acids present a well solubility and low toxicity but are not as effective in reducing NH_3 losses as strong acids, cannot maintaining stable pH conditions (Ndegwa, 2008; Hyldgaard, 2014). Recent studies settled the greatest effectiveness in manure emission mitigation, closely of 100% for sulphuric, H_2SO_4 (Ndegwa Et al. 2008; Stevens et al., 2009; Eriksen J., (2008; Kai P., 2008). NH_3 volatilization decreases below pH 7, but there is almost no measurable free NH_3 around a pH of 4.5 (Hartung and Phillips, 1994) and it is completely stopped at pH 5 in pig slurries. According to data of the Ministry of Agriculture, Food and environment of Spain (2015), the dose might be 4-6 kg H_2SO_4/t slurry to reach a pH between 5.5 and 6.0. These references are in agreement with BAT indications under Directive 2010/75/EU. It is recommended to add lime after the treatment to neutralise the pH before the application to soils.

1.3.2. Selection of the suitable additive for microorganism's inhibition

Native microorganisms of manure are responsible for bioconversion of organic matter and N-related compounds during the storage. There are available commercial products capable to reduce or even inhibit such activities. One case is compounds called statins, which are extensively used since their ingestion decreases cholesterol levels in humans and alters rumen fermentation (Bodas R., 2012). Moreover, Miller et al. (1986, 2001, 2006) reported that lovastatin, that belongs to the statins family, is a hydroxymethylglutaryl-SCoA (HMG-CoA) inhibitor that delayed CH_4 production due to the growth inhibition of *Methanobrevibacter*, a methanogen present in the rumen: the found that approximately 4 mg/L of lovastatin resulted in 50% growth inhibition. Pure forms of the inhibitor were used in Miller at al. (1986, 2001) works, but it was suggested that red yeast rice extract preparations containing lovastatin to inhibit rumen methanogens (Miller et al, 2006). The red yeast (RY) is used in Asian countries as medicine, food colouring and additive. It contains, among other compounds,

monacoline K, identical to the statin commercialized as lovastatin. Therefore, the RY is not a pure form of lovastatin; the supplier of this commercial additive suggests 20-40 mg RY/L to completely inhibit CH_4 formation in anaerobic tests. The doses used in this work were calculated taking into account that in humans RY act on fats reducing cholesterol, which are the counterpart of VFA. Thus, knowing that manure contains 0.13 g VFA/kg (Bonmatí, 2001) and the quantity of product available, for a 60% and a 30% of reduction respectively, the expected effective doses are 30 µg RY/L and 15µg/L. It has been assumed that RY dose is directly related to the VFA content in the same way as in humans for cholesterol reduction.

1.4. EXPERIMENTAL METHODS TO DETERMINE EMISSIONS

Some methodologies have been developed and validated to determine NH_3 emissions from manure and have been applied in both open and closed animal production installations. The main methods are static and dynamic chambers because their easy implementation. A high experimental area is not needed and they present low power requirements (Greatorex, 2000). When comparing different techniques, dynamic chamber represents the best option (Annex 2). It includes an air conditioning system to minimize temperature effects as well as an automated sampling system.

2. OBJECTIVES

The purpose of this work is to evaluate the effect of different manure storage strategies over N-related compounds emissions in order to achieve a high level of environmental protection. To reach this global objective the following goals are proposed:

- Evaluate the two mitigation strategies during storage: additive and acidification.
- Comparison, regarding tabulated values of IPPC (European Directive), of attained NH_3 and GHG emissions with these strategies.
- (secondary) Design and set-up of an open chamber for measuring NH_3 and GHG emissions.

3. MATERIALS AND METHODS

In the following sections the design of a non-conventional static chamber was used in order to perform a set-up of anaerobic tests to assess the addition strategies in terms of maximum gaseous emissions. Next, an open chamber was designed to measure NH_3 and GHG emissions and therefore, it was used for the suitable strategy selected in order to be closer to the reality (external rafts).

3.1. MANURE CHARACTERIZATION AND ANALYSIS

FIGURE 1 UNDERFLOOR MANURE STORAGE AT PUIGLLONG FARM

The experiment took place in IRTA (Caldes de Montbuí, Barcelona). The slurry used was produced under underfloor manure storage from Puigllong farm (Vic, Barcelona) (Figure 1).The collected manure came from 4000 heads of 60 kg fattening sows. Samples were collected in plastic containers between 60 and 25 L of capacity and stored at 4ºC.

The slurry was analysed for total ammonium nitrogen (TAN) by distillation method, Solid matter content (TS, VS), Chemical Oxygen Demand as oxidized organic matter estimation (COD), Alkalinity by titration (TA, PA), Volatile Fatty Acids (VFA) by gas chromatography; and total

Carbon, total Nitrogen and total Hydrogen content (CHN) (Table 3.). Analytical methods applied are described in Annex 3. In the same way a mixture of solid and liquid excreta of cow slurry was analysed in order to contrast the emission effects under acidification conditions (see assay 3 in section 3.3.3).

TABLE 3 MANURE CHARACTERISATION (MEAN VALUES)

PIG MANURE CHARACTERISATION (assay 1 and 2)

Date	TAN (gN/L)	pH	DQO (g/kg)	ST (g/kg)	SV (g/kg)	AT (g/L)*	AP (g/L)*	C%	H%	N%
March 17th	5.51	7.55	256.24	152.30	103.60	5.70	10.07	5.57	7.27	1.20
April 27th	5.48	7.32	211.62	147.50	102.10	5.70	10.07	-	-	-

COW SLURRY MIXTURE (assay 3)

Date	TAN (gN/L)	pH	DQO (g/kg)	ST (g/kg)	SV (g/kg)	AT (g/L)*	AP (g/L)*	C%	H%	N%
May 5th	3.20	8.65	170.01	97.30	80.30	4.00	5.40	-	-	-

*units: gCaCO3/L. - : results not finished yet.

3.2. REACTANTS

The following reactants were used:

- Commercial sulphuric acid H_2SO_4 solution 0.25 M (Scharlau, assay 1) and 0.02 M (Scharlau, assay 3), stored at ambient temperature.
- Read Yeast (RY) rice extract preparation containing lovastatin (Provect-CH4™, Provectus Environmental Products; www.ProvectusEnvironmental.com). The RY was dissolved in deionised water 0.79% NaCl to produce a RY solution of 0.30 mg/L.
- Pure powdered fumaric acid $C_4H_4O_4$ solution 0.02 M (Acros), with a maximum solubility in water, at ambient temperature, of 0.63 g/100

mLaccording to the safety data sheet of IPCS (International Programme on Chemical Safety). The product was handled with a particulate filter adapted to the concentration of the substance in air due to its irritability. Because fumaric powder particles are finely dispersed and therefore could produce explosive mixtures in the air, precautions were taken when handling the product, avoiding the deposit of powder.

- Organic acids: lactic acid $C_3H_6O_3$ solution 0.06 and 0.09 M (Fluka, 100% richness), acetic acid CH_3COOH solution 0.07 M (Scharlau, 96% richness) and propionic acid $C_3H_6O_2$ solution 0.05 M (Scharlau, 99% richness).

3.3. FACTORIAL DESIGN AND EXPERIMENTAL SET-UP

Three assays were performed to assess two mitigation strategies, acidification and RY addition. All assays and emission measurements were carried out at in triplicate, at ambient temperature (25ºC) and performed in the research institute IRTA (Caldes de Montbui, Barcelona).

3.3.1. Mitigation study (assay 1)

In assay 1, twenty-seven glass vials of 1.2 L of total volume were used with 70 g of slurry per vial (Figure 2). For the acidification conditions, and according to alkalinity properties of the control manure, 22.8 mL/vial or 13.2 mL/vial of the H_2SO_4 solution were added for pH 5.5 and for pH 6.5, respectively. Concerning RY factor, 30 µg/L or 15 µg/L of the RY solution were added in the corresponding vials. The remaining twelve vials comprised the combination of the mentioned doses for H_2SO_4 and RY. With the aim to have the same dilution in all vials, deionized water was finally added till 90 g of total media. Blanks vials (C1) were prepared adding manure and deionised water, without any additive. Each vial was hermetically closed with a butyl-rubber septum and aluminium cap; therefore, they

were bubbled with nitrogen gas N_2 (overpressure: 0.5 bars) during 1 minute and finally eliminating the bubble outlet for 20 seconds.

FIGURE 2 MITIGATION STRATEGIES SET-UP (ASSAY 1, 27 VIALS)

The aim of this experimental design was to determine if acidification and RY addition significantly mitigate GHG and/or N-related compounds emissions. The factorial design procedure was selected as experimental methodology for the first assay of emissions mitigation. This assay was designed based on a factorial design with 3 independent factors or external variables (H_2SO_4 dose, RY dose and both) at 2 levels per factor, and 3 replicates per condition (Tables 4, 5 and 6). In addition, 3 blank tests (C1-1, C1-2, and C1-3) were carried out allowing the study of emissions without treatment, as control or slurry alone. These blanks were taken as reference for comparison regarding the corresponding IPPC directive values (Annex 4).

As defined in a factorial design, all the combinations levels are analysed. This allowed selecting the best strategy as well as the maximum concentrations. Concerning vials labels ZX-Y, number X is related to the level (1: pH 5.5; 2: pH 6.5); Y denotes the replicate number and Z is related to the factor (A: H_2SO_4, L: RY). The full labels list is shown in Tables 4, 5 and 6.

TABLE 4 FACTORIAL DESIGN – ASSAY 1: ANAEROBIC CONDITION 1: ACIDIFICATION

LEVEL / FACTOR	pH 5.5	pH 6.5
H_2SO_4	A1.4 A1.5 A1.6	A2.7 A2.8 A2.9

TABLE 5 FACTORIAL DESIGN – ASSAY 1: ANAEROBIC CONDITION 2: RY

LEVEL / FACTOR	30 µg/L	15 µg/L
RY	L1.10 L1.11 L1.12	L2.13 L2.14 L2.15

TABLE 6 FACTORIAL DESIGN- ASSAY 1: ANAEROBIC CONDITION 3: RY + ACIDIFICATION

RY / H_2SO_4	30 µg/L	15 µg/L
pH 5.5	A1L1.16 A1L1.17 A1L1.18	A1L2.19 A1L2.20 A1L2.21
pH 6.0	A2L1.22 A2L1.23 A2L1.24	A2L2.25 A2L2.26 A2L2.27

3.3.2. Red Yeast (RY) dose study (assay 2)

The RY dose study was performed in the same conditions of the mitigation study. This assay was divided into two parts: low and high RY doses. In the low doses case, 30 g of slurry and the quantity of the corresponding RY dose were

FIGURE 3 HIGH AND LOW RY DOSES STUDY (ASSAY 2, 21 VIALS)

added to a glass vial of 120 mL. The applied doses were 6.0 mL RYR/vial for 30 µg/L dose level, 4 mL RYR/vial for 20 µg/L dose level, 3 mL RYR/vial for 15 µg/lL dose level, 2 mL RYR/vial for 10 µg/L dose level, 1 mL RYR/vial for 5 µg/L dose level, 0.4 mL RYR/vial for 2 µg/L dose level (Tables 7 and 8).

TABLE 7 FACTORIAL DESIGN – ASSAY 2: LOW RY DOSES

FACTOR / LEVEL	RY
0 µg/L	C1-1 C1-2 C1-3
30 µg/L	L1-3 L1-4 L1-5
20 µg/L	L2-6 L2-7 L2-8
15 µg/L	L3-9 L3-10 L3-11
10 µg/L	L4-12 L4-13 L4-15
5 µg/L	L5-16 L5-17 L5-18
2 µg/L	L6-19 L6-20 L6-21

.

Concerning high dose experiment, 20 g of manure was added in the vials of 120 mL, in addition of 6 mL RYR/vial for 30 µg/L dose level, 12 mL RYR/vial for 60 µg/L dose level, 16 mL RYR/vial for 80 µg/L dose level, 20 mL RYR/vial for 100 µg/L dose level, 30 mL RYR/vial for 150 µg/L dose level, 40 mL RYR/vial for 200 µg/L dose level (Figure 3).

In both cases, differences were compensated with addition of deionized water (identical final weights per vial) and blanks vials (C1) were prepared adding manure and deionised water, without any additive. Each vial was hermetically closed with a butyl-rubber septum and aluminium cap and bubbled with N_2 gas (overpressure: 0.5 bars) during 30 seconds and finally bubble outlet for 10 seconds.

TABLE 8 FACTORIAL DESIGN – ASSAY 2: HIGH RY DOSES

FACTOR / LEVEL	RY
0 µg/L	C1-1 C1-2 C1-3
30 µg/L	L7-3 L7-4 L7-5
60 µg/L	L8-6 L8-7 L8-8
80 µg/L	L9-9 L9-10 L9-11
100 µg/L	L10-12 L10-13 L10-14
150 µg/L	L11-15 L11-16 L11-17
200 µg/L	L12-18 L12-19 L12-20

3.3.3. Organic acids evaluation (assay 3)

Under the same conditions of the mitigation study, a new set of vials were prepared to evaluate the effectiveness of organic acids (acetic, propionic, lactic, fumaric) at pH 5.5 that was compared regarding the effect of sulphuric acid. In

this experiment, a cow slurry mixture (liquid and solid excreta with a dilution 1:1) was used to study the effects of all different acids (Table 9), while pig manure was used only to assess the effect of lactic acid, having shown the better pH measurements stability (Table 10).

TABLE 9 FACTORIAL DESIGN – ASSAY 3: EFFECT OF ORGANIC ACIDS ON COW SLURRY MIXTURE AT PH 5.5

LEVEL / FACTOR	pH 5.5
C1	C1-1 C1-2 C1-3
H_2SO_4	A1-4 A1-5 A1-6
$C_3H_6O_3$	A2-7 A2-8 A2-9
$C_3H_6O_2$	A3-10 A3-11 A3-12
$C_4H_4O_4$	A4-13 A4-14 A4-15
CH_3COOH	A5-16 A5-17 A5-18

TABLE 10 FACTORIAL DESIGN – ASSAY 3: EFFECT OF ORGANIC ACIDS ON PIG MANURE AT PH 5.5

LEVEL / FACTOR	pH 5.5
C1	C1-19 C1-20 C1-21
$C_3H_6O_3$	A2-22 A2-23 A2-24

In both cases, the alkalinity determination of both cow blend and pig manure was performed with each acid to determine to required quantity per acid to fix the pH at 5.5. According to this determination, the fumaric $C_4H_4O_4$ solution, as well as the difficulties regarding its preparation (low solubility), limited the maximum amount (48 mL) of acid per vial. To fit this limitation, the acid solutions had the following concentrations: fumaric $C_4H_4O_4$ 0.02 M; lactic $C_3H_6O_3$ 0.06 M; acetic CH_3COOH 0.07 M; propionic $C_3H_6O_2$ 0.05 M; sulphuric H_2SO_4 0.02 M.

FIGURE 4 ORGANIC ACID MITIGATION STUDY (24 VIALS)

Thus 20 g of cow slurry or 20 g of pig slurry, and 48 mL of the corresponding acid solution were added per glass vial of 120 mL. With the aim to have the same weight in all vials, weight differences were compensated with deionized water (Figure 4). Each vial was hermetically closed with a butyl-rubber septum and aluminium cap and bubbled with N_2 gas (overpressure: 0.5 bars) during 30 seconds and finally eliminating the bubble outlet for 10 seconds.

3.5. CALCULATIONS

Samples of the headspace of each vial of the mentioned three assays were taken once a week (with a syringe) to measure CH_4 and CO_2 content (see analytical methods in Annex 3, section 9.2). To evaluate the effectiveness of the strategies, the following indicators were defined:

- Reduction of N-NH_3 emissions (% of N-NH_3 emission of the blank)
- Reduction of C-CH_4 emissions (% of C-CH_4 emission of the blank).
- Reduction of C-CO_2 emissions (% of C-CO_2 emission of the blank).

The emission (kg/year) was calculated following the equation 3 (at ambient temperature 25 ºC and 1 atm), which included the gas chromatography data. This equation was adapted from the document "*Technical guide for measurement, estimation and calculus of air emissions* (2005)" that is based on IPPC and EPER inventory. This calculation facilitated the conversion to kg eqCO_2/year units and the comparison with the global potential warming data (GPW) from IPPC sources.

$$\mathrm{E}\left(\frac{kg}{year}\right)=\frac{0.04M.V_{gas\ max}.X_i.\frac{X_{N2+H2\ initial}}{X_{N2+H2final}}.10^{-3}(\mathrm{kg})}{operation\ time\ (d).0.070\ (kg\ slurry)}\ 365\left(\frac{d}{year}\right).4000\ \mathrm{sows}.\frac{5.2\ kg}{sow} \quad \text{(eq.3)}$$

Where: $V_{gas\ max}$ is the maximum volume of gas which could be generated in the hermetic vial of 1200 mL. M is the molecular weight of the gas (CO_2 or CH_4). X_i is the molar fraction of the gas measure by chromatography. X_{N2+H2} is the molar fraction of the mix of nitrogen and hydrogen gas in the vial measure by chromatography. The operation time is 43 days. The annual operation days is considered as 365. The data *5.2 kg of slurry generated per sow* was extracted from Molina (1983) and from the web page www.monografis.com.

NH_3 emissions were estimated by N mass balance, based on the initial and final total N content in each vial (see analytical method in Annex 3). The mass balance of nitrogen can be applied in non-conventional closed chambers following equations (4) to (6). Thereby, to obtain the accumulated flow as a function of time, emissions were quantified in ppm related to area and time by equation (7).

$$\text{Emission= Output – Entry} \quad \text{(eq.4)}$$

$$\int_{te}^{to} E.dt = \int_{Ce}^{C0} \mathrm{dc}.\frac{V}{10^6} \quad \text{(eq.5)}$$

$$\mathrm{E}= [(C_o - C_e).\frac{V}{10^6}]/(t_o - t_e) \quad \text{(eq.6)}$$

$$F\left(\frac{mg}{h.m^2}\right) = \mathrm{Emission}\ \left(\frac{mg}{h}\right).\frac{1}{A(m^2)} \quad \text{(eq.7)}$$

Where: "Entry" is the NH_4^+ initially in manure without treatment in a C_e concentration ($\frac{mg}{m^3}$) at t_e=0 h. "Output" is the NH_4^+ in the vial at the end of period storage, in a C_o concentration ($\frac{mg}{m^3}$), at t_o= 1032 h. "Emission" is the NH_3 emission in kg/h. "V" is the gas volume in m^3. "dc" is the accumulation of NH_4^+ inside the vial in mg/m^3. "A" is the area of the vial.

The obtained emissions were further compared with those from IPPC data bases and/or calculations (see Annex 4).

3.6. ANALYSIS OF VARIANCE

To answer the proposed conjectures, an Analysis Of Variance (ANOVA) was performed. ANOVA test is based on a null hypothesis (H_0), and an alternative hypothesis (H_1). In the first case, it wants to be tested whether the mean of each factor level (treatment) is equal for all, or if the effect of each level of factor is null. So, there is not difference between levels evaluated (eq.8). The alternative hypothesis means that at least there is some effect. The statistical regression model used is the following (eq.9).

H_0: $\mu_1=\mu_2=\ldots=\mu_{nA}$ (H_0: $\lambda_1=\lambda_2=\ldots=\lambda_{nA}=0$) (eq.8)

$Y_{ij} = \mu+\lambda_i + \beta_j+(\lambda\beta)_{ij}+\epsilon_{ijk}$; i= 1,2,….a; j=1,2,….,b; k=1,2,….,r (eq.9)

Where, Y_{ijk} : measurement; μ: overall mean; λ_i : effect of the i^{th} level factor 1; βj: effect of the j^{th} level of the factor 2; $(\tau\beta)_{ij}$: interaction effect between τ β; ϵ_{ijk}: error term due to randomness; i: quantity of levels of the factor or treatment 1; j: quantity of levels of the factor or treatment 2; k: quantity of elements taken for each of factor levels.

It has been defined a confidence interval of 95%, so a maximum error of 5% has been tolerated. The significance level (α) is thus fixed at 0.05. The p-value is compared with this 0.05 to decide if the null hypothesis (H_0) could be rejected.

Whether p-value is higher than 0.05, H_0 could not be rejected. On the contrary, if p-value is lower or equal than 0.05, H_0 could be rejected and that means that there are significant differences between the means of treatments at 5% maximum error. In the same way, if the F-calculated value is higher than the F-critic value, one or more treatments present significant statistical differences. Whether it is lower, the variation comes from treatments and from errors associated to the measures.

4. RESULTS

4.1. MITIGATION STUDY – ASSAY 1

The analytical data and the evolution of measured emissions are shown in Annexes 5 and 6. Based on these results, a reduction of 57.4% CH_4 was attained, regarding to the control, when pH was lowered to 5.5 with the H_2SO_4 solution (Table 10). No significant effect was shown for the RY addition strategy, except a slightly reduction of 2-4% in CO_2 production (Table 11). In addition, CO_2 reduction was not significant in any of the strategies, with the exception of vials with the lower RY dose (15 µg/L) combined with pH 6.5 that attained an 8% of reduction in CO_2 production (Table 11). Even if the reduction is settled to around 2-4% for any doses of RY tested, the CO_2 reduction was almost doubled (3-8%) when RY was combined with pH 6.5. In those vials with pH 5.5, the acidification prompted an increase of CO_2 emissions till 21.3%, which was reduced to 10.9 % after the addition of the lower dose of RY. *Therefore, RY addition did not mitigate CH_4 emissions but modulate CO_2 production in the case of the acidification strategy.*

Concerning the generation of the emission, the pH 5.5 strategy reached the lowest CH_4 production rate, even lower than the control. Inversely, the pH 6.5 strategy showed the highest CH_4 production rate, being similar to the control. Regarding the CO_2 production, it was higher for the pH 5.5 strategy (higher than

the control) and lower for the pH 6.5 strategy (and similar to the control). Graphics of Annex 5 show that the combination of RY addition to the pH condition level has no effect in production rates.

In this way, the ANOVA test for one factor concerning acidification proves the pH level had affected: p-value is below 0.05 and F calculated value is higher than the critic, which means that the null hypothesis could be rejected. The same ANOVA test applied to RY level shows that there has no significant effect in the emissions of CO_2 but there is in CH_4 emissions. However, the ANOVA test for two factors sets a p-value over 0.05 and F calculated value over than the critic only for the RY addition factor. So, the acidification strategy affected both CO_2 and CH_4 emissions, which is in accordance with the individual tests of one factor (Table 10).

TABLE 10 REDUCTION PERCENT ACHIEVE OF CH_4 AND CO_2 IN EACH MITIGATION STRATEGY

Vial	Mean mL CO_2	Mean mL CH_4	CH_4 reduction (%)*	CO_2 reduction (%)*	p-value***** (mL CO_2)	p-value****** (mL CH_4)
C1 (control)	390.88	821.50	-	-	-	-
A1 (pH5.5)	474.31	346.57	57.8	-21.3	0.0024**	0.00014**
A2 (pH6.5)	391.56	479.80	41.6	-0.2		
L1 (30µg/l)	376.44	791.80	3.6	3.7	0.4705**	0.0493**
L2 (15µg/l)	384.26	807.12	1.8	1.7		
A1L1	454.99	327.76	60.1	-16.4	0.6146*** 0.0013****	0.5740*** 1.87E-8****
A1L2	433.50	341.98	58.4	-10.9		
A2L1	359.76	448.59	45.4	8.0		
A2L2	368.11	449.44	45.3	5.8		

*Regarding the control. **ANOVA test of one factor. *** ANOVA test of two factors, RY factor p-value. **** ANOVA test of two factors, acidification factor p-value. *** **Annex 7, ANOVA test tables results

Results calculated from the equation (3), shown in Table 14, are in accordance with these inferences. The strategy generating less equivalent-CO_2 per year is that at pH 5.5. Comparing the emission value obtained for the control with the corresponding tabulated value from IPPC (286,298.84 kg CH_4/year; Annex 4), the data is consistent bearing in mind that the tabulated value account not only the storage phase but all steps concerning the management of the slurry.

So, even if pH 5.5 condition produces 17.6 % CO_2 emissions more than the control, there is a positive balance in terms of equivalent CO_2, being this the most effective strategy (Table 8).

TABLE 11 CO_2 EQUIVALENT EMISSIONS PER YEAR OF THE DIFFERENT MITIGATION STRATEGIES CONSIDERING ONLY THE STORAGE

	CO_2 Emissions (kg/ year)	CH_4 Emissions (kg/ year)	Total eqCO_2 (kg/ year)	p-value**** mL CO_2/d	p-value **** mL CH_4/d
C1	631	3,647	84,507	-	-
A1	766	1,538	36,151	0.0121*	4.52E-7*
A2	632	2,130	49,620		
L1	608	3,515	81,451	0.7914*	0.4044*
L2	620	3,583	83,028		
A1L1	734	1,455	34,199	0.5789** 5.77E-5***	0.8376** 6.54E-10***
A1L2	700	1,518	35,616		
A2L1	581	1,991	46,382		
A2L2	594	1,995	46,483		

*ANOVA test of one factor. ** ANOVA test of two factors, RY factor p-value. ****ANOVA test of two factors, acidification factor p-value. *** *Annex 7, ANOVA test tables results

Focusing on NH_3 emissions (Table 12), it should be noted that TAN level has not been significantly reduced after 43 operation days. The most important effect

was observed when 30 µgRY/L dose was combined with the acidification method at pH 5.5, attaining a reduction of TAN of 15% regarding to the control and mitigated a 91.7% of NH_3 emissions. Thus strategies A1L1, A1L2 and A2L1 (Table 12) contained less final TAN (measured at t=43 days) than TAN at t=0 days. Moreover, the ammonia maximum emissions calculated for the control are around 100 kg NH_3-N/year. This data is of the same order of IPPC values, set to 57.6 kg NH_3-N/year for storage in external rafts (see Annex 4).

TABLE 12 NH_3 EMISSIONS MITIGATION AND FLUX (F) AFTER 43 OPERATION DAYS

ref.	Mean TAN (g N/L) (t= 0 days, TAN=4.285 g N/L)	F mg N/(h.m2)	% TAN reduced*	Emission E kg NH_3-N/year (4000 sows)	p-value TAN
C1	4.51	1416	0.0	102.1	-
A1	4.30	116	4.5	8.4	0.6428**
A2	4.53	1574	-0.5	113.5	
L1	4.34	342	3.7	24.6	0.7023**
L2	4.54	1627	-0.7	117.3	
A1L1	3.83	-2909	15.0	-209.8	0.3508*** 0.0954****
A1L2	4.12	-1076	8.6	-77.6	
A2L1	4.25	-227	5.7	-16.4	
A2L2	4.42	851	2.0	61.4	

*Regarding the control. **ANOVA test of one factor. ***ANOVA test of two factors, RY factor p-value. **** ANOVA test of two factors, acidification factor p-value. *****Annex 5, ANOVA test tables results.

On the other hand, at the end of the assay, an increase of the pH was observed in all of the cases: the pH was 7.45 -7.84, even in the case of strategies

with an initial pH of 5.5 and 6.5. Moreover, the final pH around 7.45 was associated with a sulphate concentration increase (between 150 and 200 regarding other strategies; see Table 13). This happened only in the case of pH 5.5 condition combined or not with RY. This increase of the basicity and presence of sulphates (SO_4^{2-}) could be explain by the fact that denitrifying bacteria could have been less efficient than sulphate reducing bacteria at pH 5.5 which might prompt the sulphate reduction reactions:

$SO_4^{2-}+4\ H_2 \rightarrow H_2S+2\ H_2O+2\ OH^-$ and $SO_4^{2-}+CH_3COOH \rightarrow H_2S+2\ HCO_3^-$

In all of cases SO_4^{2-} concentration is hardly over than those measured in farmland and planted soils which are between 5 and 10 mg S-SO_4^{2-}/kg soil according to Reussi (2008).

TABLE 13 SO_4^{2-} CONCENTRATION MITIGATION STUDY, 43 OPERATION DAYS (ASSAY 1)

Ref.	SO_4^{2-} (mg/L)*
C1	39
A1	4079
A2	28
L1	19
L2	23
A1L1	3381
A1L2	3405
A2L1	27
A2L2	23

*Mean of three replicates

4.2. RED YEAST DOSE STUDY – ASSAY 2

Taking in account that the RY effect was not clear in the mitigation study (section 3.3.2.), the second assay was design to study different RY doses. Once again, the objective was to detect a significant effect of this additive in the reduction of CH_4, CO_2, and NH_3 emissions, regarding to the control (manure without additives). After 19 days, the obtained data didn´t show effects when the dose was reduced, for any of the three emissions studied (Tables 11, 12 and 13). The obtained data of assay 2 and the evolution of measured emissions are shown in Annex 7.

To verify this, ANOVA test is carried out (see Annex 7). In accordance with data, p-value is over 0.05 (Table 14) and F calculated value is lower than the critic, which means that the null hypothesis could not be rejected and there was no significant effect in the emissions of CO_2, CH_4, NH_3 and neither in the generation rate of these contaminants. Therefore RY doses between 30 and 2 µg/L did not lead to a mitigation effect (Table 15 and 16). This result was used to design a new assay to study the behaviour for doses higher than 30 µg/L that is not finished yet.

However, these results are not in accordance with assay 1 where RY addition (30 and 15 µg/L) seems modulate CO_2 production and NH_3 emissions in the case of the acidification strategy. Two possible explanations might be the duration of the assay 2, which was shorter than assay 1, or a lesser surface for the contact manure-air influenced the emissions rate generation (assay 1: 1200 mL as volume vial; assay 2: 120 mL as volume vial).

On the other hand and in view of the no significant effect observed, VFA are likely to not be related with RY doses, unlike humans. Therefore, it is suggested for future works another assay with doses of order of mg as recommended by the provider (2-200 mg RY/L).

TABLE 14 P-VALUE DATA FROM ANOVA TEST OF ONE FACTOR, RY LOW DOSES (ASSAY 2)

	p-value mL CO_2	p-value mL CH_4	p-value mL CO_2/d	p-value mL CH_4/d	p-value TAN
RY	0.5497	0.4342	0.7197	0.8704	0.5845

TABLE 15 NH_3 EMISSIONS MITIGATION AND FLUX (F) AFTER 19 OPERATION DAYS (ASSAY 2)

ref.	Mean TAN (g N/L) (t=0 days , TAN=2.755 g N/L)	F mg N/(h.m2)	% TAN reduced regarding control	Emission E kg $NH_{3\text{-}N}$/year (4000 sows)
C1	2.86	1,791	-	32.28
L1	2.91	2,644	-1.75	47.65
L2	2.93	3,042	-2.56	54.82
L3	2.91	2,587	-1.63	46.63
L4	2.94	3,155	-2.80	56.87
L5	2.87	2,018	-0.47	36.38
L6	3.02	4,520	-5.59	81.47

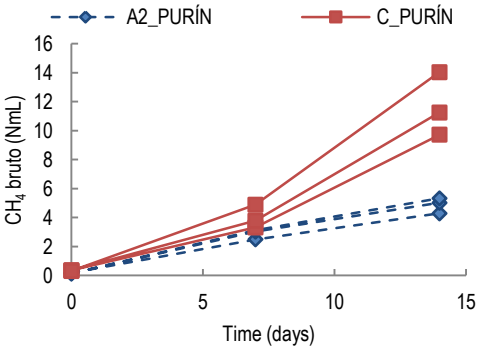
TABLE 16 CO_2 EQUIVALENT EMISSIONS PER YEAR OF THE DIFFERENT MITIGATION STRATEGIES CONSIDERING ONLY THE STORAGE (ASSAY 2)

	CO_2 Emissions (kg/ year)	CH_4 Emissions (kg/ year)	Total eqCO_2 (kg/ year)
C1	329	764	17,911
L1	341	817	19,132
L2	332	903	21,102
L3	333	905	21,152
L4	326	882	20,619
L5	346	893	20,896
L6	331	838	19,600

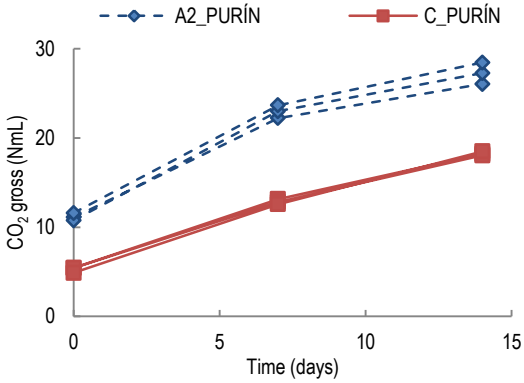
4.3. COMPARISON WITH ORGANIC ACIDS AND ITS EFFECTS ON COW SLURRY MIXTURE

As far as organic acids mitigation study, after 14 operation days, CH_4 emission was stopped (Annex 8). The addition of lactic acid reduced CH_4 emissions in pig manure regarding to the control in 58.3% (Graphic 1) but increased the emitted CO_2 in 33.3% (Graphic 2). In terms of equivalent CO_2 the balance was positive, being the CH_4 more harmfully for the environment. When comparing controls of cow mixture slurry and pig manure, CH_4 generation rate was high in the case of that of pig manure but almost the half when contrasting CO_2 generation rate (Annex 8).

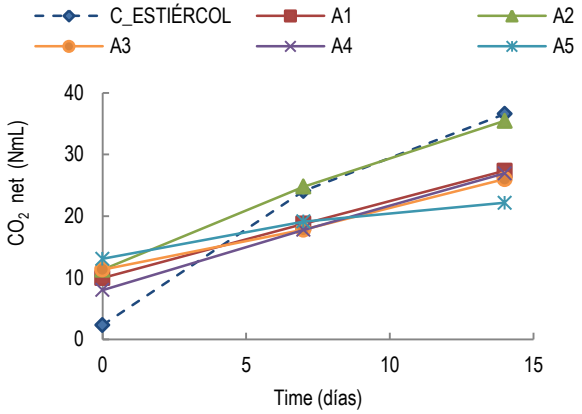
On the other hand, CO_2 emission was reduced in presence of acids, excepting the case of the lactic acid which maintained a similar CO_2 level as the control. The most effective after 14 days was the acetic acid with a reduction, regarding to the control, of 40.5%. The sulphuric acid addition reached a reduction of 27% of CO_2 emissions (Graphic 3).



GRAPHIC 1 NET GENERATION OF CH_4 FOR PIG MANURE (C_PURÍN: CONTROL C1)



GRAPHIC 2 GROSS GENERATION OF CO_2 FOR PIG MANURE (C_PURÍN: CONTROL C1)



GRAPHIC 3 NET CO_2 GENERATED DURING ORGANIC ACIDS MITIGATION STUDY FOR COW SLURRY (C_ESTIERCOL: CONTROL C1)

After 14 operation days, pH was measured (Table 17). If pH increased along the time, as occur in assay 1, not having introduced sulphates at the origin, it could be supposed that the pH increment was caused by the transformation of the detached CO_2, at the beginning when acid was introduced in the closed vial, in

carbonates (CO_3^{2-}). Therefore, as a recommendation for an extended operation period, the evolution of pH must be monitored.

TABLE 17 INTERMEDIATE PH, 14 OPERATION DAYS (ASSAY 3)

COW SLURRY MIXTURE		
Ref.	**Initial pH (t=0 days)**	**Final pH (t=14days**
C1	6.79	6.71
A1-4	5.50	5.93
A2-7	5.50	5.78
A3-10	5.50	5.91
A4-13	5.50	6.15
A5-16	5.50	6.00
PIG MANURE		
Ref.	**Initial pH (t=0 days)**	**Final pH (t=14days**
C1	7.05	6.99
A2-222	5.50	6.19

4.4. OPEN CHAMBER

This section describes the procedure and considerations (see Annex 2) for designing an open chamber. The recommended dimensions (Fig.5) for an open chamber are: a height of 20-40 cm and an area to perimeter ratio >10 cm. Therefore, to attain this recommendation, a 24.04 L plastic open-head drum with a diameter of 24.5 cm and a height of 51 cm was adapted. The adaptation of the height of this container was done by filling the excess height (21 cm) with an inert material (dry sand cover with a polyethylene bag (Ndegwa, 2008)). Therefore, the area to perimeter ratio was 15 cm and its capacity was reduced to 14.14 L. The modified container had 30 cm high.

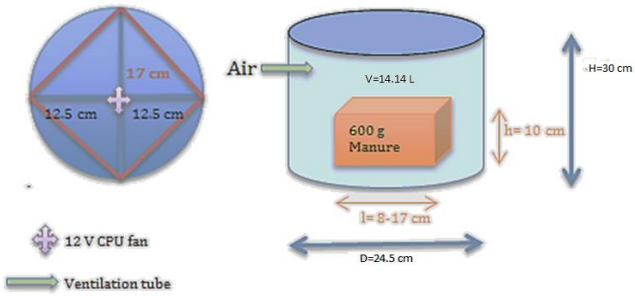


FIGURE 5 DYNAMIC CHAMBER DESIGN

The manure, placed inside the chamber, must be contained in a square plastic tray with a maximum length of 17 cm, bearing in mind the open-head drum diameter (Fig.5, left hand). Besides, this tray had a depth of 10 cm based on the consideration that dynamic chambers sinks 5-10 cm in soils. The amount of manure (70 g) that was introduced in this tray was similar to that one used in the mitigation study (see section 3.3.1.). Finally, the aeration of the chamber was ensured by a fan of 12 V (idem a CPU fan), fixed on a drum lid. This fan was connected to the chamber through a pipe (8 mm diameter, 13.5 cm length), located in the top (Fig. 5). The estimated air flow was 4 m/s (Figure 6).

FIGURE 6 DYNAMIC CHAMBER CONSTRUCION (DRUM 1: CONTROL (WIHOUT VENTILATION); DRUM 2: 2 FANS OF 12V; DRUM 3: 1 FAN OF 12V

5. CONCLUSIONS

In view of results obtain it could be concluding that:

ASSAY 1:

pH 5.5 condition has been the suitable strategy to reduce CH_4, close to 60%, presenting a lesser emission quantify in terms of CO_2 equivalents/year. This mitigation strategy allows a reduction in the generation rate of CH_4 and NH_3 emissions, which were reduced in 92% regarding the non-acidified control. The RY addition did not mitigate CH_4 emissions but modulate CO_2 and NH_3 production in the case of the acidification strategy. However H_2SO_4 addition increased SO_4^{2-} concentration to dangerous levels for farmland and planted soils. The amount of pH to 7.45-7.84 after the operation time may be due to a sulphate reducing activity (Table 18).

ASSAY 2

The RY low doses have shown no significant effects, contrary to assay 1. In this way, NH_3 emissions have increased in 67% (Table 19). A possible explanation for this result might be that the assay duration was too short or generation rate was related to the contact surface between manure and air. Besides, VFA are likely to not be related with RY doses, unlike humans. As a suggestion for future works, new RY doses with order of mg/L (2-200 mg RY/L) instead µg/L.

ASSAY 3

Organic acids were most effective than strong to stop or reduce GHG emissions in the case of cow slurry mixture, particularly the acetic acid. Related to pig manure, lactic acid showed the same mitigation effect as H_2SO_4. Moreover, cow manure was likely to emit less GHG than pig's.

TABLE 18 SUMMARY OF RESULTS ASSAY 1

	Assay 1 (t=43 days)							
	30 µg/L	15 µg/L	pH 5.5	pH 6.5	15 µg/L + pH 5.5	30 µg/L +pH5.5	15 µg/L +pH 6.5	30 µg/L + pH 6.5
CH_4 % reduction**	+	+	57.4%	41.6%	58.4%	60.1%	45.3%	45.4%
CO_2 %reduction**	4%	2%	-21.3%	+	-10.9%	-16.4%	5.8%	8%
TAN% reduction**	4%	+	4.5%		8.6%	15%	2%	5.7%
Final pH***	7.6							
SO_4^{2-}(mg/L)	x2*	x2*	x 400*	x3*	x340*	x330*	x2*	x3*
NH_3-N kg/year %reduction**	76%	-15%	92%	-11%	176%	306%	40%	116%
eqCO_2 kg/year	81	83	36	50	36	34	46	46

+: No significant effect regarding the conrol; *Number of times over SO42- concentration measured in farmland or planted soils, 10 mg/kg soil (Reussi, 2008).** Regarding the control.*** Mean.

TABLE 19 SUMMARY OF RESULTS ASSAY 2 AND 3

	Assay 2 (t=19 days)	Assay 3	(t=14 days)
	low doses RY	cow slurry blend	pig manure
CH_4 % reduction*	+	0.00%	58.30%
CO_2 %reduction*	+	40.5%***	-33.3%
TAN% reduction*	-2 to 6% **	n.m****	n.m****
Final pH	7.5**	6.0	6.0
NH_3-N kg/year %reduction*	-67%**	n.m****	n.m****
eqCO_2 kg/year	+	n.m****	n.m****

+: No significant effect regarding the control; * Regarding the control.** Mean.*** Reached for CH_3COOH, the most effective acid.**** nm: no measured.

As future work following studies are proposed:

-Set-up of low and high RY doses combined with acid at pH 5.5 to study the influence in CH_4, CO_2 and NH_3 emissions mitigation.

-Experimental study of acidification strategy with strong acids free of SO_4^{2-} to obtain a fertilizer fulfilling market conditions and which not exceeded usual concentrations of phosphorus, nitrates, sulphates or chloride of the soil.

-A study to verify the activity of sulphate reducing bacteria at pH 6.5 and SO_4^{2-} concentration generated to prove if a low pH is related to the high SO_4^{2-} concentration found at the end of the mitigation study (assay 1).

-Set-up of mitigation study with cow and pig slurry with the same proportion of liquid and solid excreta from animals subjected to the same diet, to compare generation rates of CH_4, CO_2 and NH_3.

6. REFERENCES AND NOTES

APHA, WPCF and AWWA. (2005). Standard Methods for the Examination of Water and Wastewater. 21ª ed. American Public Health Association. Washington, D.C.

Amon B., Hutchings N., Dämmgen U., Webb J. (2016). EMEP/EEA emission inventory Guidebook.

Arenas L. (2015). Diseño de cámara estática cerrada y medición de flujos de gases de efecto invernadero (GEI) en suelos. Universidad Nacional de Colombia. pp. 32-39.

Best available techniques (BAT) reference document for the intensive rearing of poultry or pigs. European IPPC Bureau (2015).

Best available techniques (BAT) reference document for the intensive rearing of poultry or pigs. European IPPC Bureau (2003).

Blanes V., Pedersen V. (2005). Ventilation flow in pig houses measured and calculated by carbon dioxide, moisture and heat balance equations. Biosystems Engineering. Vol. 92, pp. 483-493.

Bodas R., Prietoa N., García-González R.,Andrésa S.,Giráldeza F.,Lópeza S.(2012). Manipulation of rumen fermentation and methane production with plant secondary metabolites. Instituto de Ganadería de Montaña (CSIC-Universidad de León), Departamento de Producción Animal. Animal Feed Science and Technology, vol.176, pp.78– 93.

Bonmati, A. (2001). Usos de L'Energia tèrmica per a la millora del procés de digestió anaeròbia de purins de porci i per a la recuperació de productes d'interès. Tesis doctoral. Universidad de Lleida.

Burton, C.H. 1997. Manure management-treatment strategies for sustainable agriculture. Silsoe Research Institute. Bedford, UK. 181 pp.

Butterbach-Bahl K., Kiese R., Liu C. (2011). Measurements of biosphere-atmosphere exchange of CH4 in terrestrial ecosystems. Methods in Enzymology. Vol. 495, pp.271-287. ISSN 0076-6879, DOI: 10.1016/B978-0-12-386905-0.00018-8

Campos Pozuelo A.(2001).Optimización de la digestión anaerobia de purines de cerdo mediante codigestión con residuos orgánicos de la industria agroalimentaria.Universitat de Lleida (LEA).

Caracterización de las Explotaciones andaluzas del sector porcino.(2007).SECRETARÍA GENERAL DE AGRICULTURA, GANADERÍA Y DESARROLLO RURAL.

Castañeda E.O.; Sierra D.J.; Cuarón I.J.A. (1995). Lisina en función de la proteína cuando se formula a un perfil ideal de aminoácidos para cerdos en crecimiento. Memorias del VII Cobos, P M. 1989. Tecnología de ensilados. Memorias del Primer ciclo de conferencias sobre microbiología Pecuaria. Univ. Autónoma de Chapingo. pp.110.

Christiansen J., Korhonen J.,Juszczak R., Giebels M., Pihlatie M.(2011).Assessing the effects of chamber placement, manual sampling and headspace mixing on CH_4 fluxes in a laboratory experiment. Plant Soil. Vol.343, pp. 171-185.

Clough, T.J. , P. Rochette, S.M. Thomas, M. Pihlatie, J.R. Christiansen, & R.E. Thorman. (2012) Chapter 2: Chamber Design. Nitrous Oxide Chamber Methodology Guidelines. Global Reserch Alliance on Agricultural Greenhouse Gases.

Cocolo G., Hjorth M., Zarebska A., Provolo G., (2016). Effect of acidification on solid-liquid separation of pig slurry. Biosystems engineering. Vol.143, pp.20-27.

Congreso Nacional AMENA, Veracruz (1995), 2-4th November. pp. 190.

Dai X., Blanes-Vidal V., (2013). Emissions of ammonia,carbon dioxide and hydrogen sulfide from swine wastewater during and after acidification treatment; effect of pH, mixing and aeration. Journal of Environmental Management. Vol.115, pp.147-154.

Davidson E., Savage K., Vercho L.t, a Navarro R. (2002). Minimizing Artifacts and Biases in Chamber-Based Measurements of Soil Respiration. Agricultural and Forest Meteorology Vol. 113, pp.21–37.

Dourmand J.Y. (1991). Maîtrise de la charge polluante des effluents des élevages porcins. Journée Régionale EDE de Bretagne. Loudéac le 20 novembre 1991. Vol. 5.

Duarte, V.F., A. Magaña C., y F.Rodríguez G. (1990). Utilización de heces en la alimentación animal. I Caracterización químico-nutricional de heces de bovinos y porcinos. Téc. Pec. Méx. Vol.28, pp.22-29.

Eriksen J., Sorensen P., Elsgaard L., (2008). The fate of sulphate in acidified pig slurryduring storage and following application to cropped soil. J. Environ. vol. 37, pp.280–286. doi:10.2134/jeq2007.0317

EUROSTAT (2013). Agri-Environmental indicator-manure storage, Main tables and database. http//:ec.europa.eu/eurostat/statistics-explained.

FAO (2013).Mitigation of greenhouse gas emissions in livestock production.A review of technical options for non-CO_2 emissions. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS,Rome

Flotats X., Bonmatí A., Campos E., Teira M. (2000). El proceso de secado de purines en el marco de una gestión integral de residuos ganaderos.Universidad de Lleida. Departamento de Medio Ambiente y Ciencias del Suelo.Laboratorio de Ingeniería ambiental. Residuos nº53.

Flotats, X., Campos, E., Palatsi, J., Bonmatí, X. (2001). Digestión anaerobia de purines de cerdo y codigestión con residuos de la industria alimentaria. Porci; Monografías de actualidad, 65, pp 51-65.

Fundación Labein para IHOBE, S.A. (2005).Technical Guide for measurement, estimation and calculus of air emissions. Sociedad pública de gestión ambiental.pp.15-17.online:

http://www.ingurumena.ejgv.euskadi.eus/contenidos/documentacion/eprtr/es_guia/adjuntos/agroalimentario.pdf

Galloway J., Dentener F., Capone D. et al. (2004). Nitrogen cycles: past, present, and future. Biogeochemistry, vol.70, pp.153–226.

Generalitat de Catalunya Departament de Medi Ambient. Inventari d'emissions i fonts contaminants de Catalunya (EPER-CAT) criteris a aplicar per les explotacions ramaderes en la notificació de dades relatives a l'emissió de contaminants.

Gilkinson R. S.; Frost P.J.; BrowneD. J. (2015). The effects of storage time and temperature on biogas production from dairy cow slurry. Biosystsem Engineering vol. 129, pp.48 -5 6.

Gonzalez-Avalos, E. y Ruiz-Suarez, L. G. (2001). Methane emission factors from cattle manure in Mexico. Bioresource Technology vol. 80, pp. 63-71.

Greatorex, J. M. (2000). A review of methods for measuring methane, nitrous oxide and odour emissions from animal production activities. JTI - Institutet för jordbruks- ochmiljöteknik. pp.1-27.

Guidelines for measuring CH_4 and N_2O emissions from rice paddies by a manually operated closed chamber method. NIAES. (2015). Version 1, august.

Hartung J.; Phillips V.(1994). Control of gaseous emissions from livestock buildings and manure stores. Journal Agricultural Engineering Research, vol.57, pp.173–189

Hill, D.T., Jenkins, S.R., (1989). Masuring alkalinity accurately in aqueous systems containing high organic acid concentrations.

Hylgaard J., Ken. (2014). Una planta y un método para acidificar estiércol animal. European patent. ES 2 444 584 T3, pp.1-13.

Hou Y. ;Velthof L. G.; Oenema O. (2015). Mitigation of ammonia, nitrous oxide and methane emissions from manure management chains: a meta-analysis and integrated assessment. Global change biology vol .21, pp. 1293–1312.

Hutchinson G.; Mosier A.(1981).Improved soil cover method for measurement of nitrous oxides fluxes..Soil Sci. Soc. Am. J., vol. 45, pp.311-316.

Informe Semestral Fundación Guanajuato Produce. (2014). "Adopción de tecnologías para el saneamiento y reutilización de aguas residuales y subproductos generados de la actividad pecuaria en el área de influencia del D.D.R. 005 en el Estado de Guanajuato.

IPCC. (2007). IPCC fourth assessment report. Technical summary. pp. 1-74.

Kai P.; Pedersen P., Jensen P., Hansen M., Sommer S. (2008). A whole-farm assessment of the efficacy of slurry acidificacion in reducing ammonia emissions. Europ. J. Agronomy, vol.28, pp. 148-154.

Külling D.R.,Menzi H.,Kröber T.F., Neftel A, Sutter F., Lischer P., Kreuzer M. (2001).Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during the storage as affected by dietary protein content. Journal of Agricultural Science, Cambridge. Vol.137, pp. 235-250.

Liangwei D. , Yang L. , Ziai C. , Gangjin L., Hongnan Y. (2014).Separation of swine slurry into different concentration fractions and its influence on biogas fermentation. Applied Energy vol 114, pp 504-511.

Liping Z., Jing S., Yuefang Z., Liugen C., Guofeng S., Jianchu Z. (2016). Ammonia and greenhouse gas emissions from different types of deep litter used for pig rearing Livestock Science, vol.188, pp 166–173.

Liu X., Hung-Soo J., Ndegwa P.; Harrison J.(2015). Reduction of volatile fatty acids and odor offensiveness by anaerobic digestion and solid separation of dairy manure during manure storage. Journal of Environmental Management vol.152, pp.91-98.

MAGRAMA (2015). Evaluación de las técnicas de gestión de deyecciones en ganadería. Ministerio de agricultura, alimentación y medio ambiente.

Ministerio de Agricultura , Pesca y alimentación, CUADROS DE CÁLCULO DE EMISIONES DE GASES DEL SECTOR GANADERO EN RELACIÓN CON LA DIRECTIVA IPPC Registro Estatal de Emisiones y Fuentes Contaminantes (EPER-ESPAÑA).

Miller T., Meyer W. (1986). Methanogens in human and animal intestinal Tracts. Systematic and Applied Microbiology, vol.7, pp.223–229.DOI: 10.1016/S0723-2020(86)80010-8

Miller T., Meyer W. (2001).Inhibition of Growth of Methane-Producing Bacteria of the Ruminant Forestomach by Hydroxymethylglutaryl-SCoA Reductase Inhibitors.Journal of Dairy Science, vol.84, pp.1445-8.DOI: 10.3168/jds.S0022-0302(01)70177-4

Miller T., Meyer W.(2006) Control of rumen methanogenesis by inhibiting the growth and activity of methanogens with hydroxymethylglutaryl-SCoA inhibitors. International Congress Series, vol. 1293, pp.131– 137.

Molina C., Murgueitio E. (1983). Valor nutritivo del estiércol de cerdo en tres sistemas de procesamiento. Manizales.Tesis. Universidad de Caldas. Facultad de Medicina Veterinaria y Zootecnia.

Molloy S.; Tunney H. (1983). A laboratory study of ammonia volatilization from cattle and pig slurry. Irish Journal of Agricultural Research, 22, pp.37–45.

Murphy D., O'Doherty J., Pierce K., Boland T., Callan J., Lynch M.(2010). The effect of dietary benzoic acid concentration on nitrogen utilisation, manure ammonia and odour emissions in finisher pigs. University College Dublin. . doi: 10.1017-S2040470010001901

Ndegwa, P.M., Hristov, A.N., Arogo, J., Sheffield, R.E., (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. Biosyst. Eng. 100, pp. 453–469.

Oenema O., Wrage N., Velthof G., Groenigen J., Dolfing J., Kuikman P. (2005) Trends in global nitrous oxide emissions from animal production systems. Nutrient Cycling in Agroecosystems, vol.72, pp.51–65.

Osorio A.,Ferreira-Tinoco I., Gates R., Oliveira-Rocha K. ,Combatt-Caballero E. Campos F.(2013). Adaptation and validation of a methodology for determining ammonia flux generated by litter in naturally ventiled poultry houses. Dyna.pp. 1-8 DOI:10.15446/dyna.v81n187.40806

Osorio A., Ferreira-Tinoco I., Gates R., Oliveira De Paula M. , Mendes L..(2012). Evaluation of diferent methods for determinin ammonia emissions in poultry buildings and their applicability to open facilities. Dyna, vol.178, pp. 51-60. ISSN 0012-7353

Page H. L; Ni J.; Zhang H., HeberJ. A., Mosier S.N. (2015). Reduction of volatile fatty acids and odor offensiveness by anaerobicdigestion and solid separation of dairy manure during manure storage. Journal of Environmental Management, vol.152, pp. 91-98.

Pihlatie, M.., Christiansen, J., Aaltonen, H., Korhonen, J., Nordbo, A., Rasilo, T., Pumpanen, J. (2013). Comparison of static chambers to measure CH_4 emissions from soils. Agricultural and Forest Meteorology, vol.171-172, pp.124–136. http://doi.org/10.1016/j.agrformet.2012.11.008

Portejoie S., Dourmad J., Martinez J., Lebreton Y. (2004). Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs.Livestock Production Science, vol.91, pp.45–55.

Reussi N., Echevarría H., Sainz H.(2008). Estimación del contenido de sulfato en suelos del sudeste bonaerense. Unidad Integrada INTA-FCA Balcarce.vol.39, pp.23-25.

Rochette, P., Eriksen-Hamel, N.S., (2008). Chamber measurements of soil nitrous oxide flux: are absolute values reliable? Soil Science Society of America Journal 72

Rochette, P., & Hutchinson, G. L. (2005). Measurement of Soil Respiration in situ: Chamber Techniques. Micrometeorology in Agricultural Systems, pp. 247–286.

Rochette P. (2011). Towards a standard non-steady-state chamber methodology for measuring soill N2O emissions. Animal Feed Science and Technology vol.166-167, pp.141-146.

Sorensen P.; Eriksen J. (2009).Effects of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. Agriculture Ecosystems and Environment vol 131, pp.240-246.

Soto, M., Mendez, R., Lema, J.M.,(1993). Operaciones y control de digestores anaerobios (I y II). Dpto. De Ingeniería Química. Universidad de Santiago de Compostela.

Stevens R., Laughlin R.,Frost J.,(2009). Effect of acidification with sulphuric acid on the volatilization of ammonia from cow and pig slurries. doi: 10.1017/S0021859600070106.

Sutton M., Erisman J., Dentener F., Möller D. (2008). Ammonia in the environment: from ancient times to the present. Environmental Pollution, vol.156, pp.583–604.

Zhang H., (2012), Johnson G., Fram M.(2012), Managing phosphorus from animal manure. Division of agricultural sciences and natural ressources, Oklahoma State University.PSS-2249. http://osufacts.okstate.edu

Web page: http://www.adap.org.es

Air pollutant and emission factor library, web page: http://www.apef-library.fi/

Web page: http://www.fao.org

PRTR-CAT, Web page: http://dtes.gencat.cat/mediamb_prtr/AppJava/publicoConsultesPerSubstancia.do

Web page: http://www.monografias.com/trabajos82/utilizacion-excretas-fermentadas-cerdo/utilizacion-excretas-fermentadas-cerdo2.shtml

7. ACRONYMS

ADAP Asociación de Empresas para el Desimpacto Ambiental de los Purines

ANOVA Analysis of Variance

BAT Best Available Technology

BOE Boletín Oficial del Estado

C Carbon

CH_3COOH Acetic acid

CH_4 Methane

CHN Carbon-Hydrogen-Nitrogen content

$C_3H_6O_2$ Propionic acid

$C_3H_6O_3$ Lactic acid

$C_4H_4O_4$ Fumaric acid

CO_2 Carbon Dioxide

CO_3^{2-} Carbonate

COD Chemical Oxygen Demand

Cu Copper

EEA European Environment Agency

EMEP European Monitoring Evaluation Programme

EPER España Registro Estatal de emisiones y Fuentes Contaminantes

EU European Union

EUROSTAT Statistical Office of the European Communities

FAO Food and Agriculture Organization

FAOSTAT Food and Agriculture statistics

FID Flame-ionization Detectors

GC Gas Chromatography

GHG Greenhouse gas

H_0 Null Hypothesis

H_1 Alternative Hypothesis

H_2SO_4 sulphuric acid

i Quantity of levels of the factor or treatment (ANOVA)

ICSC International Programme on Chemical Safety

IPCC Panel of Experts on Climate Change

IPPC Integrated Pollution Prevention and Control

IRTA Research & Technology Food & Agriculture

j Quantity of elements taken for each of the factor levels (ANOVA)

M Molarity (mol/L)

MAGRAMA Ministerio de Agricultura, Alimentación y Medio Ambiente

N Nitrogen

N_2 Nitrogen gas

NH_3 Ammonia

NH_4^+ Ammonium

NO_x Nitrogen Oxides

N_2O Nitrous Oxide

P Phosphorus

PA Partial Alkalinity

PRTR Registro Estatal de Emisiones y Fuentes Contaminantes

RY Red Yeast

TA Total Alkalinity

TAN Total Ammonium Nitrogen

TS Total Solids

SSV Volatile Solids

VFA Volatile Fatty Acids

VOC Volatile Organic Compounds

Zn Zinc

α Significance level

β_j Effect of the j^{th} level of the factor 2 (ANOVA)

Y_{ijk} Measurement (ANOVA)

μ Overall mean (ANOVA)

λ_i Effect of the i^{th} level factor 1 (ANOVA)

$(\tau\beta)_{ij}$: interaction effect between τ β (ANOVA)

ϵ_{ijk}: error term due to randomness (ANOVA)

ANNEXES

8. ANNEX 1: CURRENT REGULATION

Table 20 shows pig manure resulting emissions ranges. In this context, the IPPC Directive from EU aims to reduce emissions from agricultural activity. Spain must communicate pollutant emissions for determining the total of them according to guidelines established by the IPPC and the European Environmental Assessment. The Ministry of Agriculture, Food and Environment should prepare the annual "Nitrogen and Phosphorus Balance in Spanish Agriculture" in order to meet the requirements of Eurostat and the latest editions of the IPCC guidelines. The most relevant regulation related to this work is carried out and classified in two levels in Table 21.

As a response to international and state-wide regulation requirements, the study of strategies delaying the emissions to the atmosphere and contributing to the reduction of the pollution problem is a necessity. Documents as document as best available techniques (BAT) for intensive rearing of poultry and pigs (IPPC, 2005), tables for the calculation of gas emission from the cattle sector according to IPPC Directive (MAGRAMA 2013, EPER-ESP), and Guidebooks related to EMEP/EEA (2013) are usually used as reference.

TABLE 20 MINIMUM AND MAXIMUM CONCENTRATION RECOMMENDED FOR GASES EMISSIONS. SOURCE: DOCUMENTATION TÉCNICA INNOVA 1412 PHOTOACUSTIC FIELD GAS-MONITOR

Gas	Low limit (mg/m^3)	High limit (mg/m^3)
NH_3	2	50
CO_2	0	10 000
CH_4	4	50
N_2O	2	30

TABLE 21 CURRENT EUROPEAN AND STATE.WIDE REGULTION RELATED TO PIG MANURE MANAGEMENT

European Regulation	Spanish regulation
DIRECTIVE 91/676 / CEE of the Council of 12th December 1991 concerning protection of waters against pollution caused by nitrates from agricultural sources. -Manure containing 170 kg N/hectare	• Royal Decree 261/1996, of 16th February -Transposition of Directive 91/676/CEE. • Royal Decree 825/2005, of 8th July About fertilizing products.
Regulation 1069/2009 SANDACH. -Categorization of animal by-products depending on their risk level to public and animal health -Disposal and use of manure (Category 2)	• Royal Decree 1528/2012, of 8th November Applicable to animal by-products and derived products not intended for human consumption. • Law 22/2011, of 28th July, Waste and Contaminated Soils. Applying to the pig slurry management. Considerate manure as waste or by-product.
DIRECTIVE 2008/120 / CE of 18th December 2008 on minimum standards for the protection of pigs. Related to the area and soil characteristics to be provided by each pig and feeding systems.	• Royal Decree 324/2000 of 3rd March 2000, laying down basic rules for the organization of pig holdings
DIRECTIVE 2010/75 / UE of the European Parliament and the Council of 24th November 2010 concerning integrated pollution prevention and control (IPPC).	Royal Decree 508/2007, of 20th April, regulating the compilation of information on emissions of the E-PRTR Regulation and integrated environmental authorizations • Decision 2017/302 of the Commission of17th February 2017 -Establishing conclusions about the BAT concerning intensive rearing from poultry and pigs.
DIRECTIVE 2008/50 / CE of the European Parliament and the Council of 21th May 2008 on environmental air quality and cleaner air for Europe. -In order to reduce harmful effects on health and on the environment	• Royal Decree 1/2001, of 20th July, -Approving the consolidated text of the Water Law. • Law 34/2007, of 15th November on air quality and protection to the atmosphere. • Royal Decree100/2011, of 28th January, -- Updating the catalogue of potentially polluting activities to the atmosphere and establishing the basic provisions for its application.

9. ANNEX 2: CHAMBERS - STATE OF ART

9.1. Closed static chamber

Closed static chamber has been the most used methodology to measure the flows of GHG in agricultural systems (Rochette, 2011). The technique is based on covering a small area of the emitting material with a hermetic chamber. This allows the exchange of gas between the floor covering the chamber and the atmosphere inside it. The increase in the concentration of the emitted gas is evaluated and quantified over time (Pihlatie et al., 2013). The sample is extracted, avoiding gas leaks using a syringe or in more sophisticated systems with vacuum pumps or automated systems.

9.2. Dynamic or open chamber

Dynamic or open chamber considers gas diffusion processes. It has a gas inlet and outlet that allows the estimation of emissions and the flow of ambient air exchanged. The concentration differences between the inflow and outflow are small requiring very precise measurement systems. Gas concentrations are analysed using infrared monitors or gas chromatography systems. The most usually used are Flame-Ionization Detectors (FID) (Gonzalez-Avalos y Ruiz-Suarez, 2001).

9.3. Comparison

When comparing different techniques for manure application, dynamic chamber techniques represent the best option (Table 22 and 23). It includes an air conditioning system to minimize temperature effects during coverage, as well

as an automated sampling system allowing simultaneous measurements and gases could be analysed by GC offering the advantage of on-line measurement.

TABLE 22 ADVANTAGES AND LIMITATIONS OF CLOSED STATIC CHAMBERS (GREATOREX, 2000)

Advantages	Limitations
Well defined system	Great spatial limitation. This can be solved by studying a larger area or with more repetitions
Low operation cost	Not applicable to all processes, such as emissions from livestock housing, or emissions from larger crops
Easy implementation and operation	Gases transport of within unvented chambers is easily impeded and are sensitive to perturbations
Suitable for studying emission processes and factorial designs comparing different emitting substances	The emissions could be studied only as a static process not as a dynamic one.
Suitable to study emissions from punctual sources or from surface	The insolation of a space alters the climatic conditions: temperature, humidity and wind speed.

TABLE 23 ADVANTAGES AND LIMITATIONS OF DYNAMIC CHAMBERS (GREATOREX, 2000)

Advantages	Limitations
Less problems associated to impediments to gas transport	It is necessary that a good mixture of the gases in the chamber is produced to avoid slanted measurements of emissions
Better quality information is obtained by taking convective transport into account	Spatial variability, difficulties of taking measurements, inability to study dynamic events
Avoiding environmental interferences	The measurement system must be very precise in order to differentiate inlet and outlet concentrations. Accurate measurement of airflow is required.
well suited to process oriented studies as static chambers	Ventilators inside the chamber lead to a major underestimation of fluxes.

9.4. Designing

To design the chamber the following points will be taken into account:

- Chamber size and morphology
- Constructive materials
- Ventilation rate

9.4.1. Dimensions and materials

The most commonly material used for chamber design are stainless steel or a plastic as Polyethylene not reacting with any of the gases (Butterbach-Bahl et al., 2011). Besides, errors associated with temperature changes inside the chamber can be avoided by using thermal insulation. (Rochette & Hutchinson et al., 2005).It is also important to establish a compromise between diameter and height. High chambers minimize pressure variations but reduce the sensitivity to measure small flows not allowing an adequate mixing of headspace air (Rochette et al., 2011) and increasing gas leaks in spite of the fact that having a greater representativeness (Davidson et al. 2002).

In this sense Rochette and Eriksen-Hamel (2008) propose to calculate an Area / Perimeter relation, which they recommend should be ≥10 cm. So, a chamber height >10 cm, but preferably between 20 and 40 cm is recommended. Taking Olensen (2013) and Külling(2001) studies as reference, dimension chamber is fixed in 25 cm of diameter and 30 cm of height.

9.4.2. Ventilation rate (air flow)

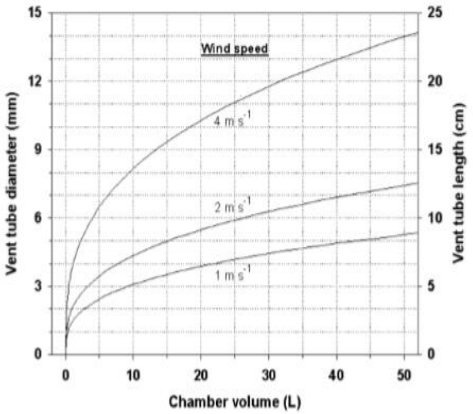
In this type of chambers, gases emissions are concentrated in the upper part and it can make difficult to obtain homogeneous samples. For this reason the use of small fans inside the chamber is recommended (Butterbach-Bahl et al. 2011). This allows reducing the effect of pressure differences, avoiding overheating and long sampling times that alter the gases flow (Christiansen et al., 2011). Kurling et

al. (2001) observed that emission rate was reduced from 25% to 12% by the introduction of two 10 volt ventilators inside the chamber.

In order to correct the additional error associated with the use of ventilation in chambers due to the sample dilution (Clough et al., 2012), Hutchinson and Mosier (1981), proposed a relation between the chamber volume, the diameter and length of the ventilation tube and the wind speed. Wind speed is recommended being between 2.5 and 4.0 m/s so as to not increase NH_3 loss (Ndegwa, 2008).Concerning air flow, the variation was established by Portejoie (2004) between 1 L/min and 5 L/min.

Taking this into account for a wind speed of 2 m/s and 15L of chamber volume the tub diameter will be 5 mm and 8.5 lengths (Figure 7). This is means the flow air required is 4.7 L/min.

FIGURE 7 DIAMETER AND LENGH OF VENTILATION TUBE FOR DYNAMIC CHAMBER (HUTCHINSON AND MOSIER 1981)



10. ANNEX 3: ANALYTICAL METHODS

In the following lines, analytical methods used to characterise the samples will be described. In all cases, three replicates were done.

10.1. Alkalinity (TA, PA, AR)

Alkalinity or basicity is the ability to neutralize an acid in the medium. It is related to the pH but it measures a different property, the buffer capacity, it means the pH resistance to be varied depending on the changes introduced in the system. This parameter is usually expressed in mM HCO_3 or mg $CaCO_3$ L^{-1}. Compounds that contribute to the alkalinity are: carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), free NH_3, phosphate (PO_4^{3-}), etc.

The standard method (2320) of Standard methods for examination of water and wastewater (1995), consists in the evaluation of the sample with a strong acid until pH 4.3. At this point more than 99% of the bicarbonate becomes CO_2. However, more than 80% of the volatile fatty acids are included (AGV Hill et al., 1987). For this reason Hill & Jenkins (1989) proposed a titration up to pH 5.75, adjusting to the actual value of alkalinity due to bicarbonate.

By taking these two pH endpoints three parameters of alkalinity measurement are defined: total alkalinity (AT) measured at the pH 4.3; partial alkalinity (AP) associated with alkalinity due to bicarbonate at the pH 5.75 and intermediate alkalinity (AI) associated with the concentration of VFA, which is the difference between AT and AP. The RA alkalinity ratio is defined as the alkali fraction due to VFA (AI) relative to total alkalinity (AT).

In this case as a strong acid, a commercial solution of H_2SO_4 0.5 N is used. For solid or pasty samples such as the manure used, alkalinity is determined in the liquid fraction of an extract. For this, 40 mL of sample were centrifuged in vials of 50 mL at 6000 rpm for 10 minutes and the pH of a known amount of the supernatant was measured. 10 mL are enough to submerge the diaphragm of the

pH-meter to be submerged in the sample. Taking into account the proposed experimental design the alkalinity was established for the following pH points: 7.00, 6.5, 5.75, 5.5 and 4.3.

For the purpose of calculation the alkalinity is expressed in units of calcium carbonate:

$$\text{AT (mg CaCO}_3) = \frac{V_{4.3} \cdot N_{H2SO4}}{V_{sample}} 50 \quad \text{(eq.6)}$$

$$\text{AP= Alkalinity due to Bicarbonate (mg CaCO}_3 L) = \frac{V_{5.75} \cdot N_{H2SO4}}{V_{sample}} 50 \text{(eq.7)}$$

$$\text{AI=Alkalinity due to VFA (mg CaCO}_3/L) = \text{AT-AP} \quad \text{(eq.8)}$$

The coefficient 50 allows expressing the alkalinity in mg $CaCO_3$/L. When there is a coefficient of 60, the alkalinity is expressed in mg acetic /L, which is useful for expressing the intermediate alkalinity.

$$\text{AI= Alkalinity due to VFA (mg Acetic}/L) = \frac{AT-AP}{50} 60 \quad \text{(eq.9)}$$

$$\text{RA(Alkalinity Ratio)} = \frac{V_{4.3} - V_{5.75}}{V_{4.3}} \quad \text{(eq.10)}$$

10.2. Biogas composition (CH_4, CO_2, H_2, H_2S)

It is important to quantitatively analyse methane (CH_4) and carbon dioxide (CO_2) contained in the biogas from the anaerobic digestion of organic matter to determine the emissions of these GHG during the storage. The method used for this is based on the chromatographic separation of the compounds from the gaseous mixture due to the affinity of each of them for the stationary phase. The detection is carried out by TCD (thermal conductivity detector) and by comparison with an external standard. The equipment used consists of a VARIAN CP-3800 gas chromatograph with two on-column injectors (Mod. 1041) and two TCD detectors (FRONT / MIDDLE).

The method is first selected using Galaxie® software. In this case FRONT_2013 where air (H_2-N_2-O_2), methane (CH_4), carbon dioxide (CO_2) and possible traces of hydrogen sulphite (H_2S) can be determined. A sample volume of 0.2 mL is then extracted from the gaseous phase of the vials (head space) through a septum which ensures the tightness of the chromatographic syringe with overflow and closing valves. It is injected into the septum of the FRONT inlet of the chromatograph. The quantification of the samples is performed by linear regression in comparison with sample of known concentration (calibrated). These data contain the following information: compound name, retention time (min), peak height (uV), area (uV.Min and% A). From the obtained areas and interpolating the linear regression line the program Galaxie®, generates the biogas composition results. These results are done in mole or mole fraction sample for each compound.

10.3. COD (Chemical Oxygen Demand)

The COD is a globalizing parameter. It is the amount of potassium dichromate, expressed in terms of mg O_2/L, consumed by a sample during a boiling process in acid medium. It indicates the amount of oxygen necessary for the oxidation of the chemical compounds contained in a medium: Organic compounds (soluble or suspended, biodegradable or not) R oxidizing minerals compounds. The method is based on the estimation of the oxidizing organic matter from the amount of oxygen required to oxidize the organic material of a sample under specific conditions of temperature, time and oxidizing agents. The range of application of the method has been established in two ranges: the low range of 500 to 12,000 mg O_2 / kg and the high range of 5,000 to 65,000 mg O_2 / kg. The necessary reagents are as follows: 0.5 N potassium dichromate, Sulphate silver, 1% solution in H_2SO_4 acid; H_2SO_4, 95-97%; Hydrogen phthalate potassium:

(as standard in COD calibration and control solution); Anhydrous, extra pure magnesium sulphate.

Procedure:

To prepare solid dilutions a known amount of magnesium sulphate and sample is weighed in a porcelain crucible depending on the expected COD concentration. For an expected COD of about 200,000 mgO2 / kg, weigh 0.2 g of sample and 2.0 g of magnesium sulphate (1:11 dilution). It is then mixed and passed through the mortar until it is well homogenized. Then, 0.05 g of this already homogenized sample is taken and completed to 0.20 g with Milli Q water (0.15 g).Add 3.6 mL of 0.5N $K_2Cr_2O_7$ and 3.6 mL Ag_2SO_4 (working in the high range).

A blank (0.1 g of magnesium sulphate completed at 0.2 g with Mili Q water) and three standards will be made.

All samples, blank and controls are placed in the digester for 2 hours at 150 ° C. After this time less to cool to room temperature. The remaining unreduced $K_2Cr_2O_7$ is determined spectrophotometrically at a wavelength of 605 nm. Oxidizing organic matter is calculated in terms of equivalent oxygen. The COD results are calculated by interpolating in the calibration line obtained with the phthalate standards using the following equation:

$$y = a + bx$$

Where, y is the absorbance value measured in the spectrophotometer; a is the ordinate at the origin (the interception); b is the slope of the line; x is the variable, in this case the concentration of COD in mgO_2 / kg in the tube.

The calculation should be corrected by subtracting the blank concentration and considering the exact weight of the sample in the tube to obtain the COD concentration of the sample in mgO_2 / kg.

10.4. Nitrogen (TAN)

Total ammonium nitrogen (N-NH_4^+) is determined by distillation method and titration. The analysis is based on the conversion of the ammonium ion (NH_4^+) in ammonia (NH_3), in liquid medium, in the presence of a base such as sodium hydroxide (NaOH, 40% w / v). NH_3 is distilled off by collecting NH_4^+ in a known and excess volume of boric acid (2% w / v) to form ammonium borate. The titration of the borate ion is carried out using hydrochloric acid of 0.1 mol / L. The NH_4^+ present initially in the sample is thus quantified. The range of application of the method is set between 50-10,000 mg / L.

To distil the samples the necessary grams are weighed and taken to a Kjeldahl digestion tube. For solid or pasty samples as manure, 0.3 to 1 g are required. The distillate is collected on the fixing solution of boric acid and titrated with hydrochloric acid 0.1 N with an automatic titrator. The volumes and conditions used during distillation are the follows: 15 mL of deionized water; 15 mL 40% NaOH; 50 mL boric acid 2%; The following are the conversion factors to be used to express the results in mg N-NH_4^+/L:

$$N - NH_4^+(\text{mg}/L) = 14{,}000.\frac{[(V_{HCl})_{sample} - (V_{HCl})_{blank}].N_{HCl}}{V_{sample}(mL)} \quad \text{(eq.11)}$$

Where, V_{HCl} (mL): volume of hydrochloric acid consumed in the titration of the blank or of the sample. N_{HCl} (N): Normality of hydrochloric acid. V_{Sample} (mL): Distilled sample volume.

10.5. Solids (TS, VS)

Total solids (ST) are the solids present in the medium, both in suspension and dissolved:

-Total volatile solids.

-Total non-volatile or fixed solids

Volatile solids (SV) are a first approximation of the organic matter content of a sample. Process:

A well-homogenized sample is weighed into a porcelain crucible tared. In the case of solid samples, 5-10 grams are taken. Dry to constant weight in an oven at 105 ° C (24 h). Allow to cool and record the weight (C). Total Solids (ST) will be obtained. The sample is then calcined at 550 ° C in the muffle for three and a half hours. Record the weight (D). The difference in weight between ST and ash is the weight due to volatile solids (SV).

$$\text{ST (\% w)} = \frac{\text{ST (g)}}{\text{100 g sample}} = \frac{(C-A)}{(B-A)}.100 \qquad \text{(eq.12)}$$

$$\text{SV (\% w)} = \frac{\text{SV (g)}}{\text{100 g sample}} = \frac{(D-A)}{(B-A)}.100 \qquad \text{(eq.13)}$$

Where, A: Crucible weight (g); B: Crucible weight + sample (g); C: Crucible weight + dry residue at 105 ° C (g); D: Crucible weight + residue at 550 ° C (g).

10.6. Volatile Fatty Acids (VFA)

The determination of volatile organic acids (VFA) in slurry is used as control parameters of anaerobic digestion. The determined acids are: acetic, propionic, iso and n-butyric, iso and n-valeric, iso and n-caproic and heptanoic acids.

The method is based on gas phase chromatography and is applicable to quantitatively and qualitatively analyze VFA of liquid and semi-liquid samples. The range of application of the method depends on the analyte considered. It is established between 10-2,000 mg / L.

Gas chromatography is a separation method in which the components of a mixture are distributed between two phases: the stationary phase, which contains a very large exposure surface, and the mobile phase, which is a gas that circulates in contact with the stationary phase. Once the sample is injected and vaporized, the chromatograph detector produces an electrical signal proportional

to the amount of matter as each compound is separated. This signal is sent to a recording and integration system that generates an intensity chart as a function of time (chromatogram). Each Gaussian peak corresponds to one component of the sample. The integrator or control software calculates the area of each peak, which is proportional to the amount of analyte.

Process (Method described for a dilution of 50%):

Centrifuge the sample at 3,500 rpm in a 10 mL tube for 5 min. Take 750 µl of the supernatant, place it in an Eppendorf tube and add 750 µl of Mili-Q-water. Centrifuge at 13,000 rpm for 10 min. Take 900 µl of diluted sample and place in a chromatography vial. Add 100 µl of formic acid to the chromatography vial and cover. Place the vials inside the sampling cart and program the automatic injection of the samples.

The results are calculated by interpolating in the calibration line obtained from the standards:

$y = a + bx$

Where, y is the value of the chromatographic peak area divided by the area of the PI; b is the slope of the line; x is the variable, analyte concentration divided by the concentration of PI (which is 1), in µg /mL.

This calculation should be corrected by subtracting the blank concentration from the sample and considering the dilution. The concentration of the different analytes is obtained in µg / mL (ppm).

11. ANNEX 4: EMISSION CALCULATION METHODS FROM IPPC

According to the tables of calculation of gas emissions of pig sector under IPPC Directive (MAGRAMA 2013) and for the State Register of Emissions and Pollutant Sources (EPER-SPAIN), the Tables 24, 25 and 26 show the calculated NH_3 and GHG yearly emissions expected using emission factors.

TABLE 24 NH_3 EMISSONS DUE TO SLURRY MANAGEMENT FOR SOWS IN CLOSED CYCLE (CODE SNAP 97-2: 1005)

nº of heads A	Volatilization barn (kg NH_3-N)		Volatilization external Storage (kg NH_3-N)		Volatilization due to fertilization (kg NH_3-N)	
	B	C = A x B	D	E = D x A	F	G = F x A
4,000	20.3442	81,376.8	14.4007	57,602.8	8.6361	34,544.4

TABLE 25 NITROUS OXIDE EMISSIONS DUE TO SLURRY MANAGEMENT FOR SOWS IN CLOSED CYCLE (CODE SNAP 97-2:1005)

nº of heads A	Nitrous Oxide Emission during the storage (kg N_2O-N)		Nitrous Oxide Emission during fertilization (kg N_2O-N)	
	H	I = H x A	J	K = J x A
4,000	0.021601	86.404	0.3239	1,295.6

TABLE 26 CH_4 EMISSIONS DUE TO SLURRY MANAGEMENT FOR SOWS IN CLOSED CYCLE (CODE SNAP 97-2: 1005)

nº of heads	A	4,000
SV Excretion (kg)	B	1,185.14
Specific Weigh of CH_4 (kg/m^3)	C	0.67
Potential Production of CH_4 ($m^3/kgVS$)	D	0.45
Conversion Factor of provincial CH_4 (Girona)	E	0.20031
Emission Factor (kg CH_4 /head)	F = (BxCxDxE)	71.51
Emission of CH_4 (kg CH_4	G = A x F	286,298.84

12. ANNEX 5: MITIGATION STUDY RESULTS

TABLE 27 ASSAY 1 - ANOVA TEST OF 1 FACTOR: ACIDIFICATION STRATEGY (PH 5.5 AND 6.5), CO_2 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	28.94	2	14.47	19.58	0.0024	5.14
Intra-groups	4.44	6	0.74			
Total	33.38	8				

TABLE 28 ASSAY 1 - ANOVA TESTOF 1 FACTOR; ACIDIFICATION STRATEGY (PH 5.5 AND 6.5), CH_4 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	237.24	2	118.62	55.17	0.00014	5.14
Intra-groups	12.90	6	2.15			
Total	250.14	8				

TABLE 29 ASSAY 1 - ANOVA TEST OF 1 FACTOR: RY ADDITION STRATEGY (30 AND 15 µG/L), CO_2 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	0.72	2	0.36	0.86	0.471	5.14
Intra-groups	2.52	6	0.42			
Total	3.24	8				

TABLE 30 ASSAY 1 - ANOVA TEST OF 1 FACTOR: RY ADDITION STRATEGY (30 AND 15 μG/L), CH_4 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	5.72	2	2.86	5.18	0.049	5.14
Intra-groups	3.32	6	0.55			
Total	9.04	8				

TABLE 31 ANOVA TEST OF 2 FACTORS: RY ADDITION AND ACIDIFICATION STRATEGY, CO_2 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
RYR	0.62	2	0.31	0.52	0.6146	4.46
Acidification	31.36	4	7.84	13.16	0.0014	3.84
Error /Total	4.77 /36.74	8 /14	0.60			

TABLE 32 ANOVA TEST OF 2 FACTORS: RY ADDITION AND ACIDIFICATION STRATEGY, CH_4 EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
RYR	0.28	2	0.14	0.60	0.5740	4.46
Acidification	235.68	4	58.92	253.05	1.88E-8	3.84
Error /Total	1.86 /237.82	8 /14	0.23			

TABLE 33 ASSAY 1 - ANOVA TEST OF 1 FACTOR: ACIDIFICATION STRATEGY (PH 5.5 AND 6.5), ML CO_2/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	13808	2	6904	10.07	0.0121	5.14
Intra-groups	4114	6	686			
Total	17922	8				

TABLE 34 ASSAY 1 - ANOVA TEST OF 1 FACTOR: ACIDIFICATION STRATEGY (PH 5.5 AND 6.5), ML CH_4/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	360068	2	180034	387.80	4.52E-7	5.14
Intra-groups	2785	6	464			
Total	362853	8				

TABLE 35 ASSAY 1 - ANOVA TEST OF 1 FACTOR: RY STRATEGY (PH 5.5 AND 6.5), ML CO_2/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	314	2	157	0.24	0.7914	5.14
Intra-groups	3868	6	645			
Total	4182	8				

TABLE 36 ASSAY 1 - ANOVA TEST OF 1 FACTOR: RY STRATEGY (PH 5.5 AND 6.5), ML CH_4/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	1323	2	662	1.06	0.4044	5.14
Intra-groups	3757	6	626			
Total	5080	8				

TABLE 37 ANOVA TEST OF 2 FACTORS: RY ADDITION AND ACIDIFICATION STRATEGY, ML CO_2/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
RYR	189	2	94	0.59	0.5789	4.46
Acidification	20564	4	5141	31.90	5.77E-5	3.84
Error /Total	1289 /22042	8 /14	161			

TABLE 38 ANOVA TEST OF 2 FACTORS: RY ADDITION AND ACIDIFICATION STRATEGY, ML CH_4/D EMISSIONS

Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
RYR	74	2	37	0.18	0.8376	4.46
Acidification	482245	4	120561	588.99	6.54E-10	3.84
Error /Total	1638 /483957	8 /14	205			

TABLE 39 ASSAY 1 - ANOVA TEST OF 1 FACTOR: ACIDIFICATION STRATEGY (PH 5.5 AND 6.5), TAN

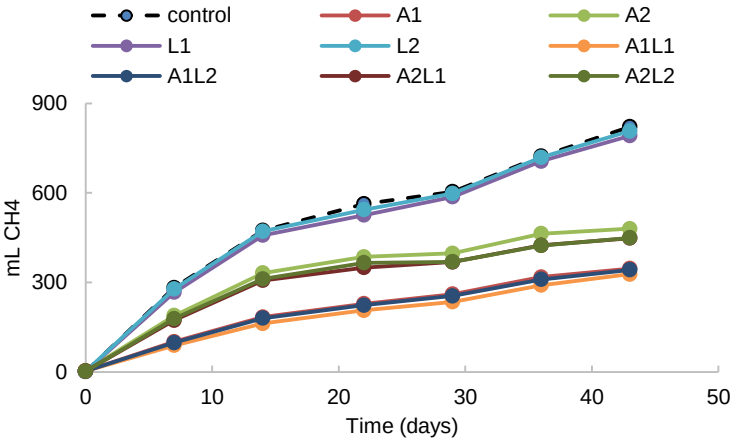
Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	0.09	2	0.05	0.48	0.6428	5.14
Intra-groups	0.59	6	0.10			
Total	0.68	8				

TABLE 40 ASSAY 1 - ANOVA TEST OF 1 FACTOR: RY STRATEGY (PH 5.5 AND 6.5), TAN

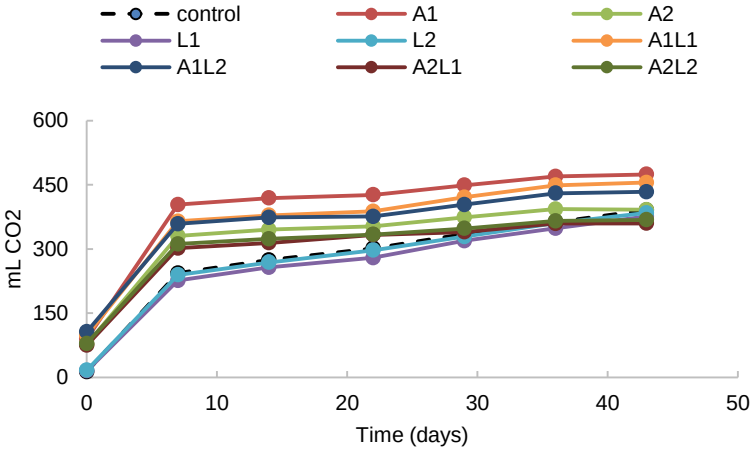
Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
Inter-groups	0.07	2	0.03	0.38	0.7023	5.14
Intra-groups	0.56	6	0.09			
Total	0.63	8				

TABLE 41 ANOVA TEST OF 2 FACTORS: RY ADDITION AND ACIDIFICATION STRATEGY, TAN

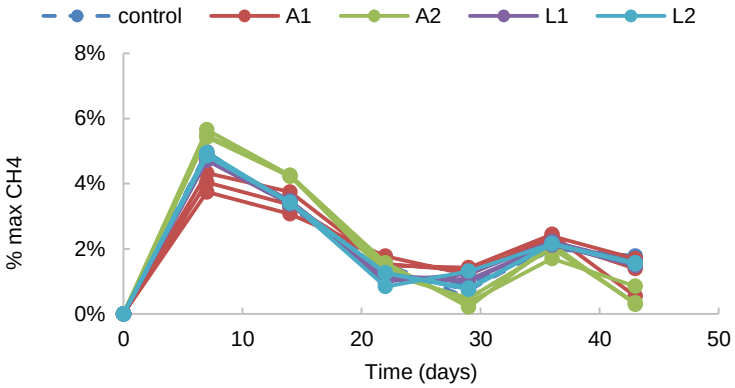
Source of variation	*Sum of squares*	*freedom degrees*	*Mean of squares*	*F Calculated value*	*p-value*	*F Critic value*
RYR	0.19	2	0.09	1.20	0.3508	4.46
Acidification	0.85	4	0.21	2.87	0.0954	3.84
Error /Total	0.59 /1.62	8 /14	0.07			



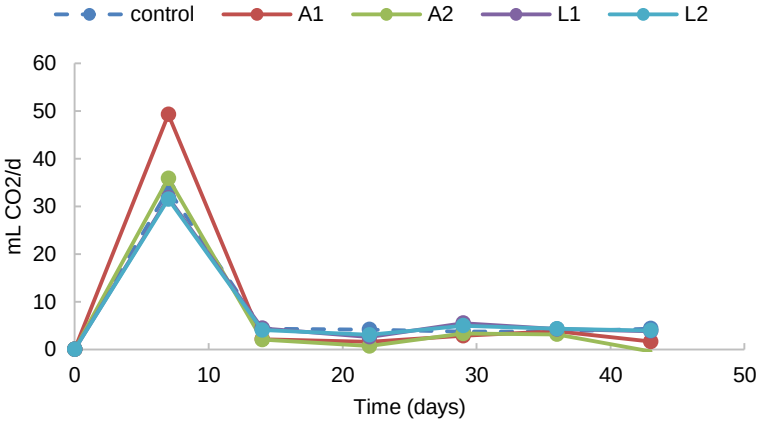
GRAPHIC 4 CH_4 EVOLUTION ALONG THE TIME IN ASSAY 1



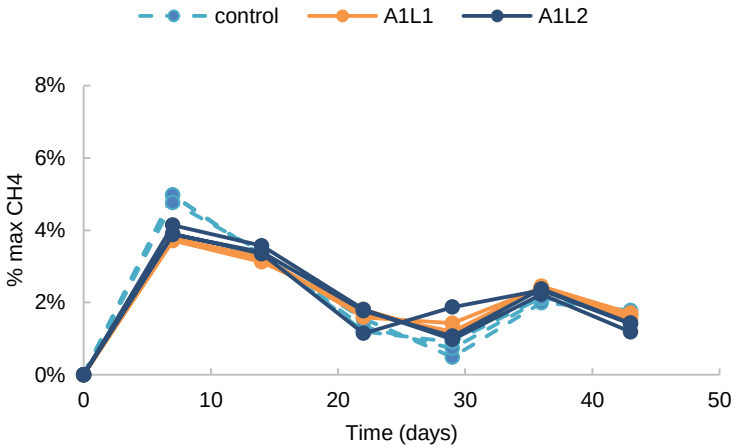
GRAPHIC 5 CO_2 EVOLUTION ALONG THE TIME IN ASSAY 1



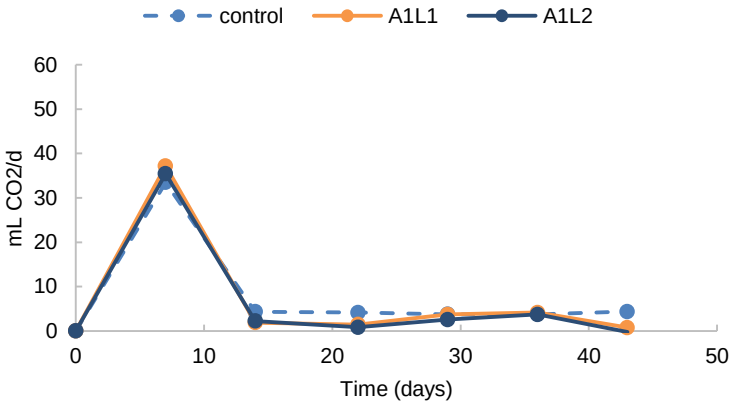
GRAPHIC 6 MAXIMUM PERCENT OF CH_4 (ACIDIFICATION & RY STRATEGY)



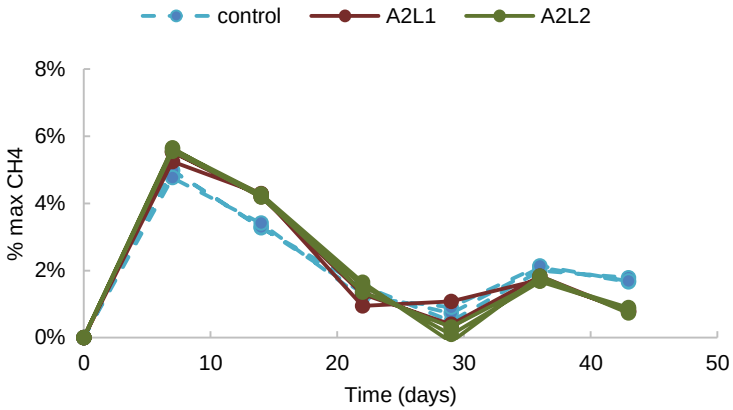
GRAPHIC 7 CO_2 PRODUCTION RATE (ACIDIFICATION & RY STRATEGY)



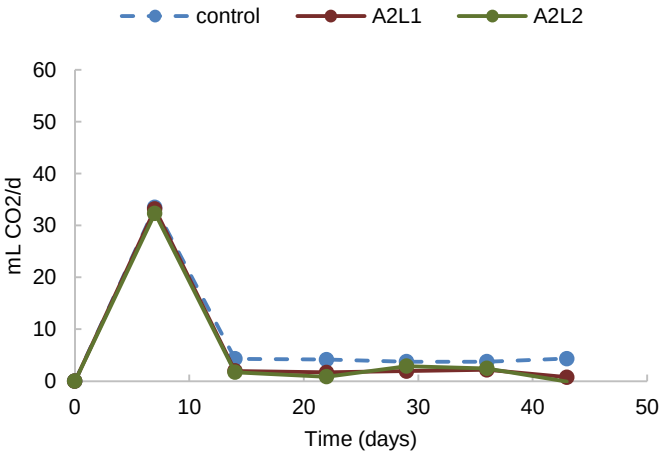
GRAPHIC 8 MAXIMUM PERCENT OF CH_4 AT PH 5.5 WITH DIFERENT DOSES OF RY, 30 µG/L (L1) AND 15 µG/L (L2)



GRAPHIC 9 CO_2 PRODUCTION RATE AT PH 5.5 WITH DIFERENT DOSES OF RY, 30 µG/L (L1) AND 15 µG/L (L2)



GRAPHIC 10 MAXIMUM PERCENT OF CH_4 AT PH 6.5 WITH DIFERENT DOSES OF RY, 30 µG/L (L1) AND 15 µG/L (L2)



GRAPHIC 11 CO_2 PRODUCTION RATE AT PH 6.5 WITH DIFERENT DOSES OF RY, 30 µG/L (L1) AND 15 µG/L (L2)

13. ANNEX 6: MITIGATION STUDY, VIALS CONTENT CHARACTERISATION

TABLE 42 CHEMICAL CHARACTERISATION AT THE END OF THE MITIGATION STUDY (ASSAY 1)

Ref.	COD (mg/kg)	desvest	Error (%)	TS (%) mean	desvest	Error (%)	VS (%) mean	desvest	Error (%)	TAN (g N/L)	SO_4^{2-} (mg/L)	pH final	pH initial
C1-1	75093	7890	0	8.93	0.28	3	5.99	0.10	2	5.097	49	7.8	7.55
C1-2	74737	1202	0	9.32	0.80	9	6.16	0.42	7	4.139	45	7.84	7.55
C1-3	85136	840	1	10.26	0.44	4	6.65	0.10	2	4.285	24	7.84	7.55
A1-4	93278	2624	3	8.44	0.84	10	5.29	0.17	3	4.254	5130	7.45	5.5
A1-5	87184	1001	1	9.02	0.34	4	6.05	0.06	1	4.190	3513	7.45	5.5
A1-6	89863	8735	10	8.32	0.22	3	5.64	0.20	4	4.467	3594	7.57	5.5
A2-7	99674	5391	5	9.18	0.89	10	6.14	0.29	5	4.630	39	7.77	6.5
A2-8	93675	4226	5	8.97	0.82	9	5.81	0.18	3	4.518	22	7.75	6.5
A2-9	96753	4139	4	7.65	0.72	9	5.03	0.19	4	4.447	22	7.76	6.5
L1-10	82163	2638	0	8.47	0.35	4	5.79	0.09	2	4.347	19	7.8	7.55
L1-11	82708	7869	0	9.15	0.77	8	6.34	0.62	10	4.325	19	7.79	7.55

Ref.	COD (mg/kg)	desvest	Error (%)	TS (%) mean	desvest	Error (%)	VS (%) mean	desvest	Error (%)	TAN (g N/L)	SO_4^{2-} (mg/L)	pH final	pH initial
L1-12	77915	6656	9	8.15	0.09	1	5.56	0.05	1	4.345	19	7.84	7.55
L2-13	70703	1808	0	7.6	0.42	6	5.06	0.23	5	4.472	19	7.8	7.55
L2-14	92764	1099	0	6.67	0.20	3	3.78	0.33	9	4.668	32	7.8	7.55
L2-15	74473	360	0	8.22	0.14	2	5.49	0.01	2	4.480	19	7.84	7.55
A1L1-16	85659	6171	7	6.31	0.62	10	4.15	0.38	9	3.684	4099	7.45	5.5
A1L1-17	53220	338	1	5.16	0.50	10	3.35	0.29	9	3.895	2938	7.47	5.5
A1L1-18	68030	5661	8	7.68	0.19	2	5.04	0.05	1	3.913	3105	7.5	5.5
A1L2-19	88582	3988	5	6.16	0.61	10	4.08	0.38	9	4.423	3101	7.59	5.5
A1L2-20	48359	876	2	6.94	0.17	3	4.48	0.16	4	3.985	3639	7.55	5.5
A1L2-21	80268	7775	1	6.62	0.22	3	4.47	0.13	3	3.944	3474	7.52	5.5
A2L1-22	48779	4732	0	6.65	0.33	5	4.56	0.27	6	4.228	35	7.74	6.5
A2L1-23	52436	1231	0	6.88	0.06	1	2.9	0.23	8	4.423	25	7.74	6.5
A2L1-24	46177	2690	6	7.36	0.40	5	5.09	0.30	6	4.099	21	7.72	6.5
A2L2-25	54143	4176	0	6.31	0.30	5	4.3	0.15	4	4.433	22	7.75	6.5
A2L2-26	51864	2932	0	5.48	0.30	6	3.55	0.12	3	4.493	21	7.76	6.5
A2L2-27	53996	1491	3	6.23	0.15	2	4.28	0.13	3	4.330	25	7.84	6.5

14. ANNEX 7: ANOVA TEST, RY ADDITION, DOSES UNDER 30 µG/L (ASSAY 2A)

TABLE 43 RESULTS OF RY LOWER DOSES STRATEGY (19 OPERATION DAYS, ASSAY 2A)

ASSAY 2	Ref.	TAN g N/L	mL CO_2	mL CH_4	mL CO_2/d	mL CH_4/d
control	C1-1	2.79	66	67	2.9	5.4
C1	C1-2	2.93	37	29	1.3	2.2
	C1-3	2.86	40	36	1.6	3.1
level L1	L1-3	2.85	42	38	1.7	3.2
	L1-4	2.90	39	33	1.5	2.7
30 µg/L	L1-5	2.98	39	33	1.4	2.2
level L2	L2-6	2.96	38	36	1.4	2.6
	L2-7	2.90	39	40	1.6	3.2
20 µg/L	L2-8	2.94	40	40	1.7	3.3
level L3	L3-9	2.96	39	39	1.6	2.9
	L3-10	2.84	41	41	1.8	3.3
15 µg/L	L3-11	2.92	39	39	1,7	3.3
level L4	L4-12	2.97	37	36	1.4	2.4
	L4-13	3.01	39	40	1.6	3.0
10 µg/L	L4-15	2.84	39	37	1.6	3.2
level L5	L5-16	2.84	41	35	1.7	2.5
	L5-17	2.88	40	40	1.6	3.0
5 µg/L	L5-18	2.90	41	39	1.7	3.1
level L6	L6-19	2.77	38	33	1.2	1.6
	L6-20	3.09	39	38	1.6	2.9
2 µg/L	L6-21	3.20	40	36	1.5	2.4

TABLE 44 ANOVA TEST: TAN DATA AFTER 19 OPERATION DAYS, LOW DOSES OF RY ADDITION (ASSAY 2A)

Source of variation	Sum of squares	freedom degrees	Mean of squares	F Calculated value	p-value	F Critic value
Inter-groups	0.05	6	0.008	0.80	0.5845	2.85
Intra-groups	0.15	14	0.010			
Total	0.20	20				

TABLE 45 ANOVA TEST: CO_2 GENERATED (ML) AFTER 19 OPERATION DAYS, LOW DOSES OF RY ADDITION (ASSAY 2A)

Source of variation	Sum of squares	freedom degrees	Mean of squares	F Calculated value	p-value	F Critic value
Inter-groups	192.11	6	32.02	0.86	0.5497	2.85
Intra-groups	524.12	14	37.44			
Total	716.23	20				

TABLE 46 ANOVA TEST: CH_4 GENERATED (ML) AFTER 19 OPERATION DAYS, LOW DOSES OF RY ADDITION (ASSAY 2A)

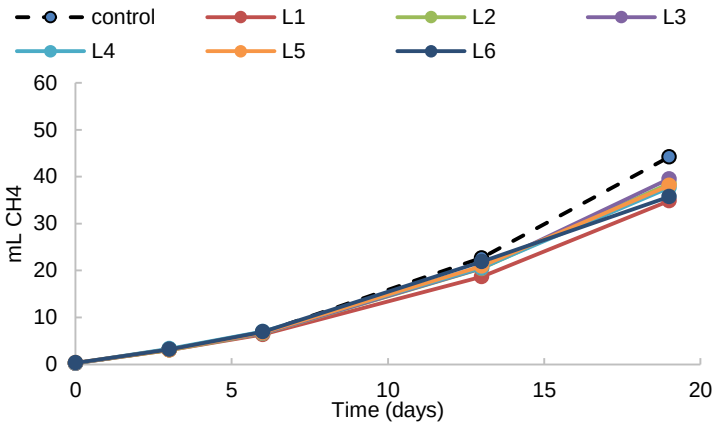
Source of variation	Sum of squares	freedom degrees	Mean of squares	F Calculated value	p-value	F Critic value
Inter-groups	164.93	6	27.49	0.43	0.8441	2.85
Intra-groups	886.24	14	63.30			
Total	1051.17	20				

TABLE 47 ANOVA TEST: GENERATION RATE OF CO_2 (ML/D) AFTER 19 OPERATION DAYS, LOW DOSES OF RY ADDITION (ASSAY 2A)

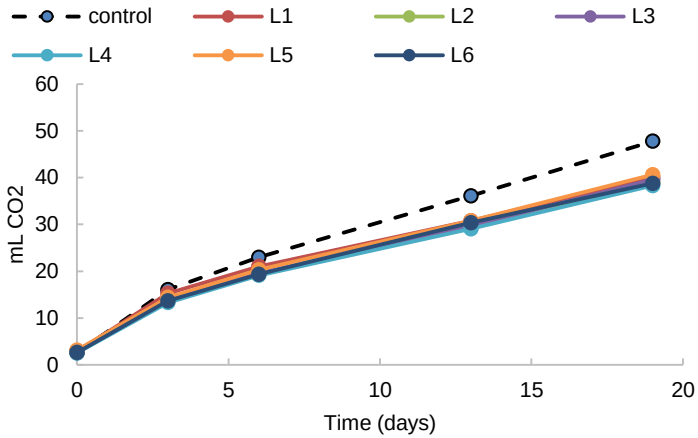
Source of variation	Sum of squares	freedom degrees	Mean of squares	F Calculated value	p-value	F Critic value
Inter-groups	0.51	6	0.09	0.72	0.6406	2.85
Intra-groups	1.65	14	0.12			
Total	2.16	20				

TABLE 48 ANOVA TEST: GENERATION RATE OF CH_4 (ML/D) AFTER 19 OPERATION DAYS, LOW DOSES OF RY ADDITION (ASSAY 2A)

Source of variation	Sum of squares	freedom degrees	Mean of squares	F Calculated value	p-value	F Critic value
Inter-groups	2.83	6	0.47	0.87	0.5401	2.85
Intra-groups	7.57	14	0.54			
Total	10.40	20				

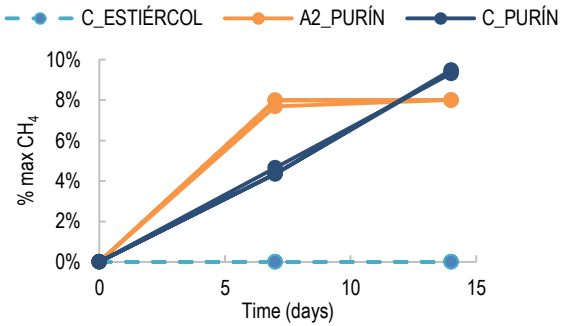


GRAPHIC 12 CH_4 GENERATION IN RY LOWER DOSES STRATEGY

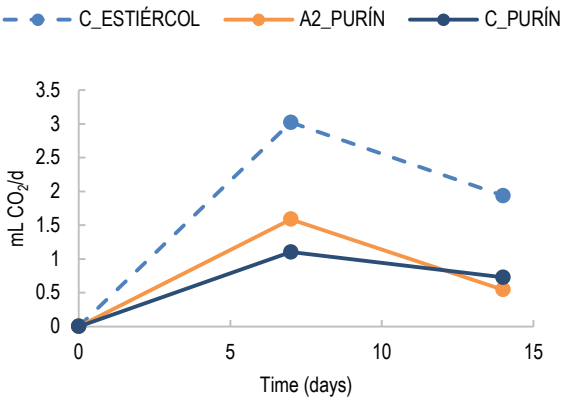


GRAPHIC 13 CO_2 GENERATE IN RY LOWER DOSES STRATEGY

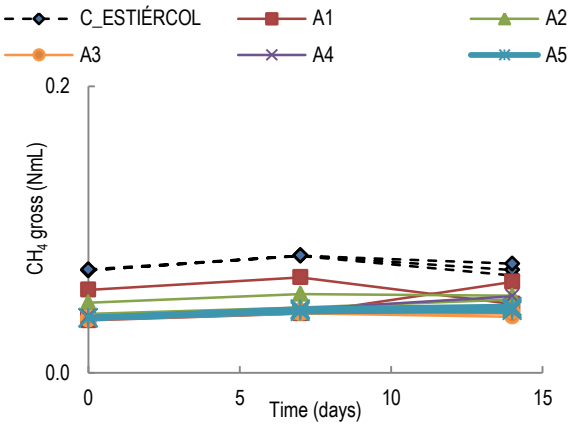
15. ANNEX 8: ORGANIC ACIDS MITIGATION STUDY (ASSAY 3)



GRAPHIC 14 GENERATION RATE OF CH_4 FOR PIG MANURE (C_ESTIERCOL: COW SLURRY CONTROL C1; C_PURÍN: PIG MANURE CONTROL C1)



GRAPHIC 15 GENERATION RATE OF CO_2 FOR PIG MANURE (C_ESTIERCOL: COW SLURRY CONTROL C1; C_PURÍN: PIG MANURE CONTROL C1)



GRAPHIC 16 GROSS CH_4 PRODUCED FOR COW MIXTURE SLURRY (C_ESTIÉRCOL: CONTROL C1)