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Mid-term report on GHG and other air emissions reduction solutions

Deliverable D2.1

INNOVATIVE DECISION-MAKING TOOL FOR DEFINING THE MOST SUITABLE MANURE MANAGEMENT STRATEGIES TO ACHIEVE A SUSTAINABLE LIVESTOCK FARMING SYSTEM DURING THE WHOLE VALUE CHAIN

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1. EXECUTIVE SUMMARY

The NUTRITIVE Project focuses on developing a decision-support tool to guide stakeholders across the manure management chain in selecting the most suitable mitigation technologies. This tool will be powered by experimental data generated through the evaluation of technologies aimed at reducing pollutant emissions. Mitigating greenhouse gas (GHG) emissions from livestock manure is highly relevant, given the significant environmental impacts associated with livestock production, particularly with respect to gaseous emissions. Within the NUTRITIVE framework, eight mitigation technologies targeting pollutants derived from livestock manure have been proposed. Deliverable D2.1 outlines the progress achieved across these technologies up to the end of the first reporting period (M18). These eight technologies were selected from different stages of the manure management chain, covering housing-level interventions, primary treatment, post-treatment processes, and land-application systems.

At housing-level interventions, NUTRITIVE project includes First Point Control (S/T1) and Dietary Interventions (S/T2). Fist Point Control strategy is focussed on the new strategies and compounds to reduce the NH_3 emissions. Therefore, this strategy includes the recycled solid manure recycle strategies for enhancing the animal health. The current advances include the in-vitro manure characterization and product screening. Current findings show that pasteurization, composting, and proper bedding management; especially maintaining adequate dry matter, humidity, and temperature; are key to limiting pathogen growth and ensuring hygienic recycled manure solids. Within the dietary interventions, CECOAGRO allows the NH_3 emissions mitigation thought dietary modification to improve the protein assimilation. The current stage of this technology includes the cobalt form addition to enhance fibre fermentation more effectively than cobalt glucoheptonate, likely due to greater bioavailability or improved microbial transport. While isoacids do not significantly affect methane production, their combined supplementation with cobalt forms offers a synergistic strategy to boost fibre degradation and microbial protein synthesis while partially mitigating the associated increase in methane yield.

As a primary treatment pathway, the project includes anaerobic digestion (AD) enhanced with the addition of biochar (S/T3) or zeolite (S/T4). These additives improve the performance of AD systems by reducing methane emissions from the resulting digestate. Moreover, both biochar and zeolite exhibit high ammonia-adsorption capacity, thereby lowering NH_3 emissions during digestate handling. Current results demonstrate that the incorporation of specific compounds can increase biogas production efficiency while contributing to overall emission mitigation.

For post-treatment, NDN and PN-ANAMMOX were selected as representative technologies for ammonia-rich wastewater effluents. Within the NUTRITIVE Project, these systems were chosen for their potential to reduce treatment-related emissions, particularly nitrous oxide (N_2O). During the first phase of the project, system setup and GHG-emission monitoring protocols were successfully implemented.

At the soil-application stage, the project assesses manure fertigation strategies and the use of additives applied to manure prior to land application. During the first 18 months, field site selection, experimental design, and GHG-measurement protocols have been completed. The upcoming farm campaign will allow full execution of the planned experiments, focusing on the capacity of land-management practices to reduce GHG emissions from applied manure.

Across all stages of the manure management chain, the project demonstrates significant potential for reducing greenhouse gas emissions through targeted technological interventions. As the next

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phase advances, empirical results from field-scale applications will be key to validating the effectiveness and scalability of these mitigation strategies. At the end of the project, these results will be reported in the Deliverable 2.2. as Final Report of the GHG emission reduction technologies.

2. INTRODUCTION

Livestock farming is a key sector that involves 40 % of the total agricultural activity in Europe. representing a total value for products equal to € 170 billion. However, there is an increasing concern due to livestock farming's contribution to environmental pollution since it generates more than 1.4 billion tonnes/year of manure leading to significant greenhouse gases (GHG) and air pollutants emissions (NH3, NOX) as well as to soil and water contamination caused by hazardous manure chemicals and biological contaminants (called here emerging contaminants). In this context extensive effort has been carried out for years to assess the detrimental effects of farming systems and to develop abatement methods to be implemented. However, despite major advancements, many fundamental issues are beyond the scope of existing legislation.

The main objective of NUTRITIVE is to develop a decision-making tool (DSS, decision support system) able to define the most efficient and sustainable (in its three pillars: environmental, economic, and social) manure management strategies for a given livestock farm limiting manure air emissions as well as soil and water contaminants. This will allow for the formulation of technical guidelines and recommendations that will support policy makers with enhanced knowledge to establish requirements for future European policies.

To fulfil this objective, the project is divided into six work packages (WP): WP1 Up-to-date inventory; WP2 Novel management strategies/technologies investigation; WP3 Modelling and Life Cycle Assessment (LCA); and WP4 Guidelines formulation; WP5 Communication, dissemination, and exploitation; WP6 Management (Figure 1).

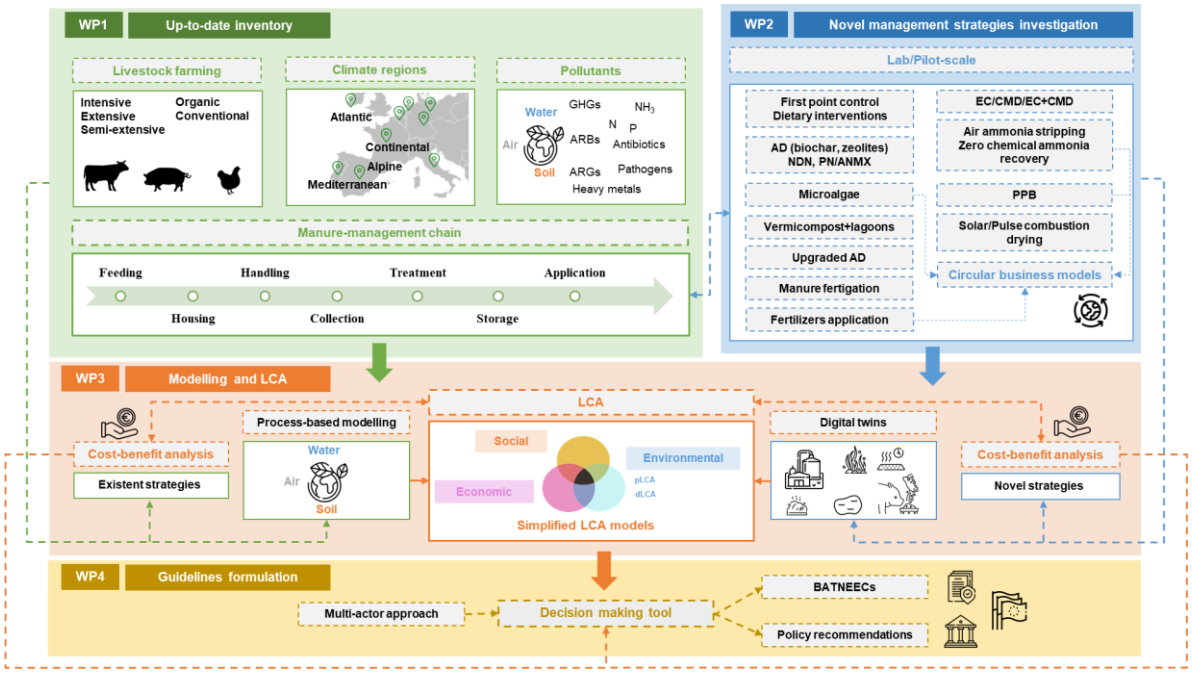

Figure 1. NUTRITIVE methodology.

NUTRITIVE anticipates a wide spread of the project outcomes, with the synthesis of the consortium as a baseline: 22 partners (4 Chinese) from 8 different countries across Europe, covering 6 climatic regions (2 Chinese ones), representing the whole supply chain experts, from animal feed to soil application.

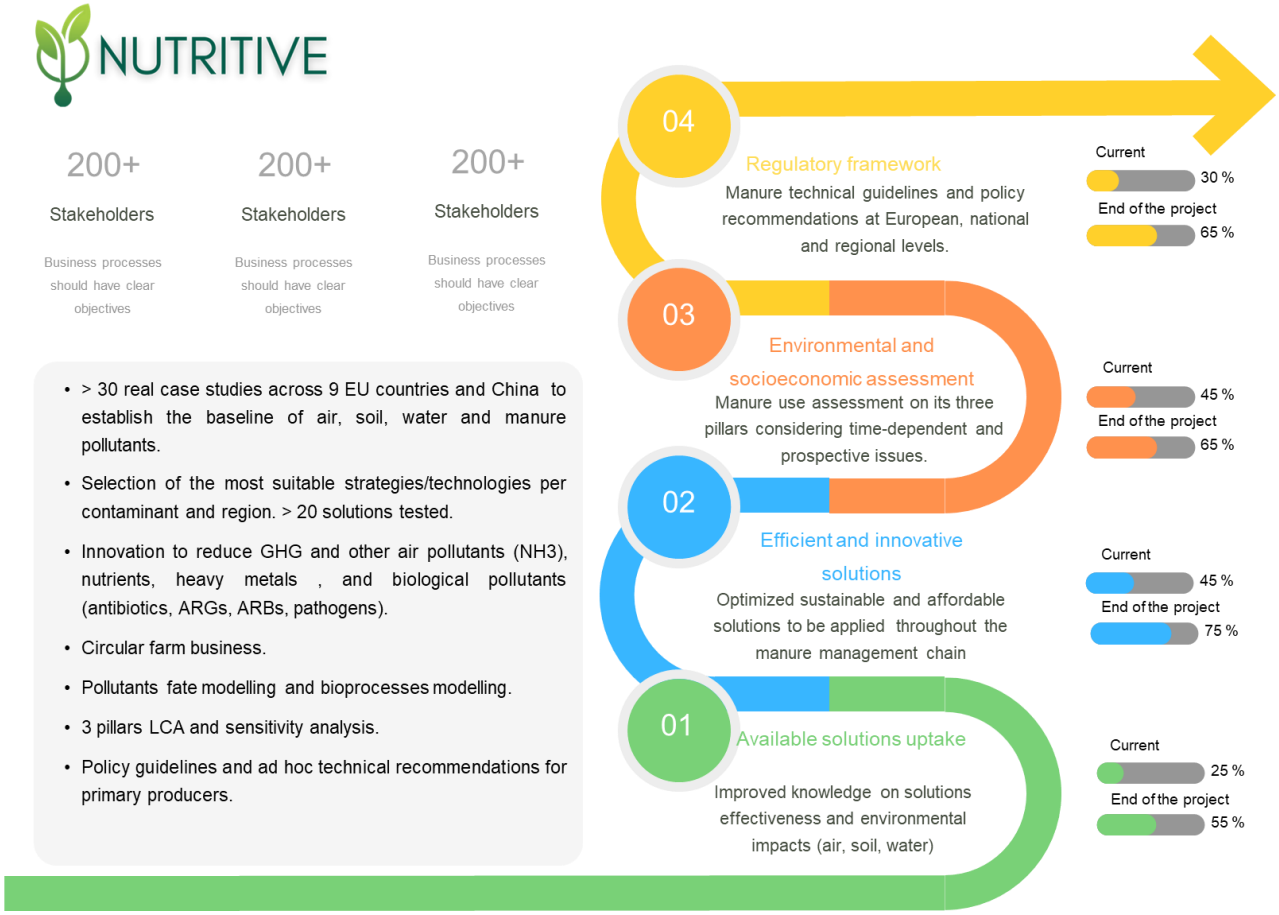

Figure 2. NUTRITIVE overview.

3. GHG emissions reduction strategies at the livestock housing level

3.1. S/T1: First point control

3.1.1. Introduction

3.1.1.1. Background.

Livestock production systems are significant contributors to global greenhouse gas (GHG) emissions, accounting for approximately 14.5% of anthropogenic emissions worldwide [Gerber et al., 2013]. Ammonia (NH_3) emissions, primarily originating from manure, represent a critical environmental and health concern. Ammonia volatilization contributes to air pollution, acidification, and eutrophication, while also serving as a precursor for secondary particulate matter formation [Bouwman et al., 2002; Sutton et al., 2011].

Current mitigation strategies have largely focused on manure management during storage and land application phases [Montes et al., 2013]. These approaches include covered storage, slurry acidification, and optimized spreading techniques. While effective in reducing emissions at later stages, these strategies overlook a critical window: the period immediately following manure excretion, when emissions begin and animals remain in close contact with the substrate. This oversight represents a significant knowledge gap in emission control research.

3.1.1.2. Knowledge Gap and Health Implications.

Freshly excreted manure initiates ammonia volatilization almost instantly, driven by urea hydrolysis and microbial activity [Sommer et al., 2006]. In confined housing systems, this results in elevated ammonia concentrations in the microenvironment surrounding the animals. Chronic exposure to high ammonia levels has been associated with respiratory disorders, reduced feed intake, and compromised immune function in livestock [Donham et al., 2002; Cambra-López et al., 2010]. Consequently, addressing emissions at the “first point” of contact is not only an environmental imperative but also a matter of animal welfare and productivity.

3.1.1.3. The First Point Control (FPC) Initiative.

The *First Point Control* initiative aims to fill this gap by developing innovative strategies to reduce ammonia emissions from fresh manure while it remains in contact with animals. Unlike conventional approaches, FPC emphasizes early intervention, targeting the biochemical and microbial processes responsible for ammonia release immediately after excretion. The initiative encompasses multiple species—ruminants, swine, and poultry—reflecting the diversity of manure characteristics and housing systems.

A distinctive feature of FPC is its dual-phase development framework. The first phase involves in vitro screening of potential strategies, including chemical additives, enzymatic inhibitors, and novel compounds designed to suppress ammonia activity or alter manure ph. Promising candidates will then advance to the second phase: validation under commercial farm conditions. This step ensures that interventions are not only effective in controlled environments but also practical and scalable in real-world settings.

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3.1.1.4. Integration of Recycled Manure Solids (RMS).

In addition to fresh manure management, FPC addresses the use of recycled manure solids (RMS) as bedding material in ruminant systems. RMS offers sustainability benefits by reducing reliance on external bedding resources; however, it poses challenges related to ammonia emissions and microbial contamination [Leach et al., 2015]. The initiative seeks to develop treatments that mitigate these risks, thereby enhancing both environmental performance and animal health outcomes.

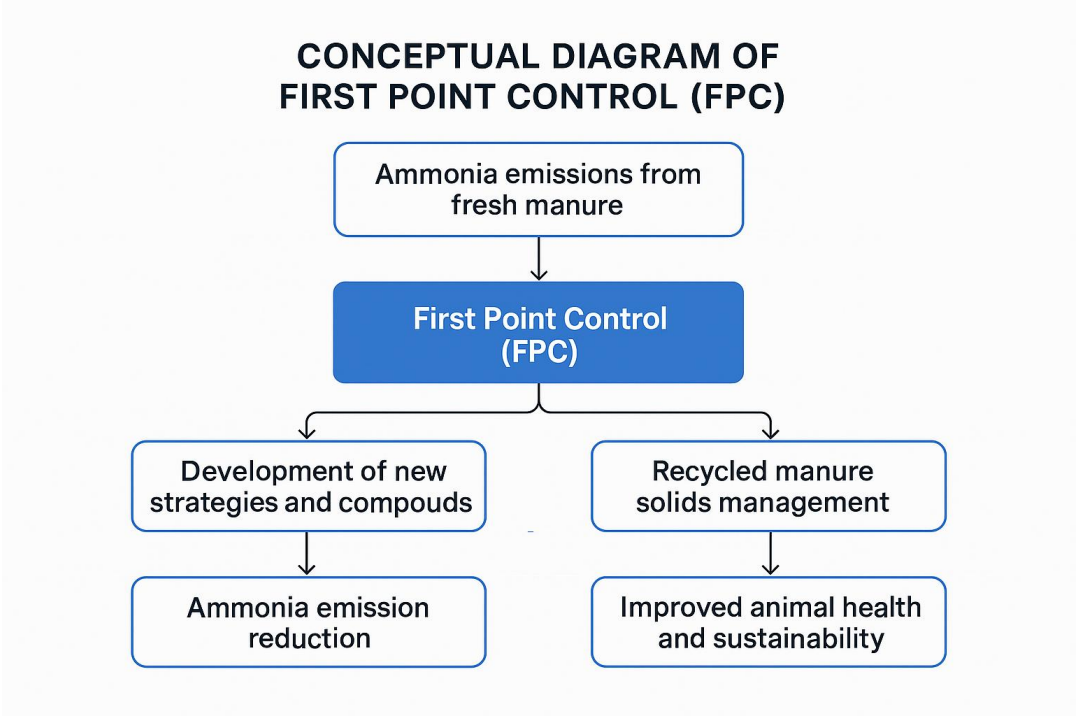
3.1.1.5. Significance and Broader Impacts.

By targeting emissions at their source, FPC has the potential to deliver multiple benefits. Reduced ammonia volatilization contributes to improved air quality and compliance with environmental regulations. Enhanced animal health translates into better feed efficiency, lower morbidity, and reduced nutrient excretion, creating a positive feedback loop for sustainability. Ultimately, this initiative aligns with global efforts to reduce the environmental footprint of livestock production while safeguarding animal welfare.

3.1.1.6. Objectives

The overarching objective of FPC is to develop and validate strategies that minimize ammonia emissions from fresh manure in contact with animals. Specific goals include:

1. Screening potential compounds and interventions in vitro for efficacy in reducing ammonia release.
2. Evaluating the top-performing candidates under commercial farm conditions across ruminant, swine, and poultry systems.
3. Developing complementary approaches for RMS management to reduce emissions and microbial risks.



3.1.2. Methodology

3.1.2.1. Experimental Design

The research was structured into two distinct phases: an **in vitro development and screening phase** and a **commercial farm validation phase**. This dual approach was designed to ensure that strategies identified as effective under controlled laboratory conditions could be validated in real-world production environments.

3.1.2.2. In Vitro Phase

The in vitro phase comprised two major components: the development of a novel ammonia measurement system and the subsequent evaluation of candidate strategies for emission reduction. The system was designed to allow continuous monitoring of ammonia emissions using a DOL-based* sensor platform. Initial efforts focused on the characterization of this system, which included determining emission dynamics within the proposed container setup, assessing the interaction between the manure substrate and the online measurement system, and optimizing airflow and container volume to maintain ammonia concentrations below the calibration threshold of 100 ppm [Sommer et al., 2006].

Calibration was performed using reference ammonia compounds to ensure measurement accuracy. Following system characterization, the next step involved adapting manure volumes to maintain emissions within the calibrated threshold. This adaptation was critical to ensure that experimental conditions remained consistent and within the operational limits of the measurement system.

Once the system was optimized, candidate compounds were selected based on theoretical properties such as their ability to modify pH, inhibit bacterial growth, or otherwise interfere with ammonia volatilization processes. Experimental treatments included either different compounds at a fixed dosage or varying dosages of the same compound, expressed as milligrams per unit volume of manure. Surface area ratios were standardized across all treatments to maintain comparability.

* DOL 53 is an ammonia sensor specifically designed for continuous measurement of ammonia (NH3) concentration in livestock houses. The sensor can accurately measure the level of ammonia in both low and high concentration and has a negligible cross sensitivity to other gases.

3.1.2.3. Recycled Manure Solids (RMS) Evaluation

In parallel, the study addressed the management of recycled manure solids (RMS), which are commonly used as bedding material in ruminant systems. RMS samples were collected from commercial herds and characterized for baseline microbial counts. Subsequent experiments evaluated bacterial population dynamics under varying humidity levels and contamination scenarios, expressed as the percentage of manure added to the bedding mix. Particular attention was given to pathogens associated with mastitis, including *Staphylococcus* spp. and *Streptococcus* spp. [Leach et al., 2015]. The most promising compounds identified in the manure emission studies were then tested for their efficacy in reducing both ammonia emissions and microbial counts in RMS.

3.1.2.4. Commercial Farm Phase

The second phase of the study involved validating the top-performing strategies under commercial farm conditions. Trials were conducted across ruminant, swine, and poultry systems to assess the practical applicability of the interventions. Parameters measured included ammonia emissions,

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animal health indicators (such as respiratory scores and feed intake), and overall system performance.

Table 1. Summary of Experimental phases.

Phase	Description	Species	Parameters Measured	Techniques
In vitro (System Development)	Characterization of ammonia measurement system; calibration and optimization	N/A	Ammonia emissions	Continuous DOL system
In vitro (Manure Emission Studies)	Testing compounds at different dosages; control vs treatments	Ruminants, Swine, Poultry	Ammonia emissions	Continuous DOL system
In vitro (RMS Evaluation)	Characterization of RMS; bacterial evolution under humidity and contamination	Ruminants, Poultry	Microbial counts (Staph, Strep, coliforms, molds)	Culture-based assays, MIC/MBC
Commercial Farm Trials	Validation of best candidates from in vitro phase	Ruminants, Swine, Poultry	Ammonia emissions, animal health indicators	On-farm gas monitoring, health scoring

3.1.2.5. Analytical Techniques

Ammonia emissions were quantified using a continuous monitoring system based on DOL technology, calibrated to a maximum threshold of 100 ppm [Sommer et al., 2006]. Microbial analyses included traditional culture-based methods for enumerating total bacterial counts and specific pathogens, as well as MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) assays to evaluate the antimicrobial properties of candidate compounds [CLSI, 2018].

3.1.2.6. Statistical Analysis

Data was analyzed using mixed-effects models to account for repeated measures and hierarchical experimental structures. Treatment effects were assessed using ANOVA, with significance set at $p < 0.05$. All analyses were performed using R as a statistical software package, and replicates were included for each treatment to ensure robustness [Montes et al., 2013].

3.1.3. Results

The results will be included as abstracts are prepared sequentially for communication purposes. Below are the results indicated under the initial abstract proposed for the FPC strategy. Further abstracts will be included in the first quarter of 2026 to reflect the finish work on the screening already performed on cattle manure and omitted here until the data analysis is finished. The following shows

the characterisation of recycled manure solids and a study of the behaviour of their bacterial load over time in different environmental conditions.

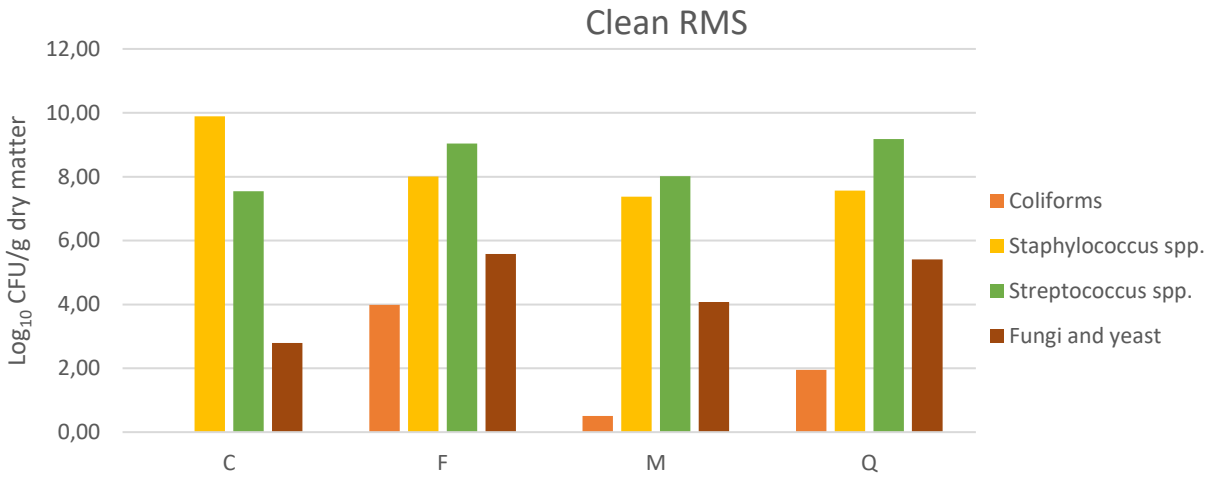

Figure 1. Left: Bacterial counts for each group of organisms in clean RMS, expressed as Log10 CFU/g dry matter. Right: Bacterial counts for each group of organisms in used RMS, expressed as Log10 CFU/g dry matter.

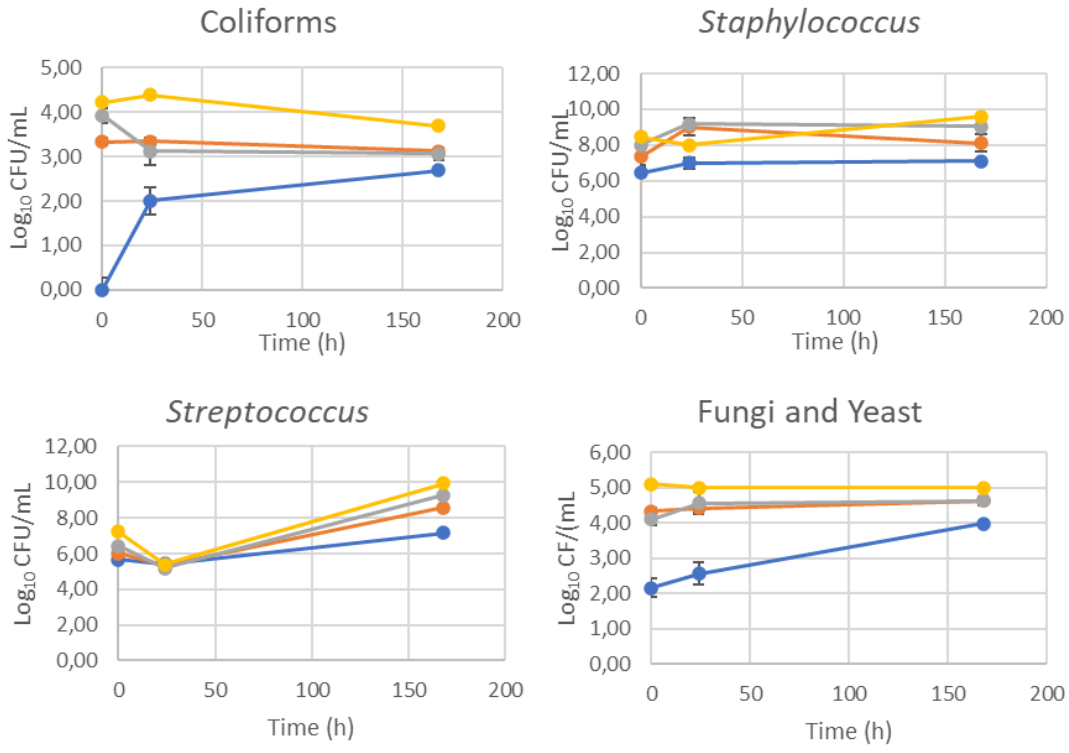

Figure 2. Bacterial counts for each group of organisms in RMS with varying percentage of inoculum inclusion (fresh manure), expressed as Log10 CFU/g dry matter. The inoculum percentage tested were Control with only RMS (blue) and the combinations RMS-inoculum in a relation of 75%-25% (orange), 50%-50% (grey) and 25%-75% (yellow).

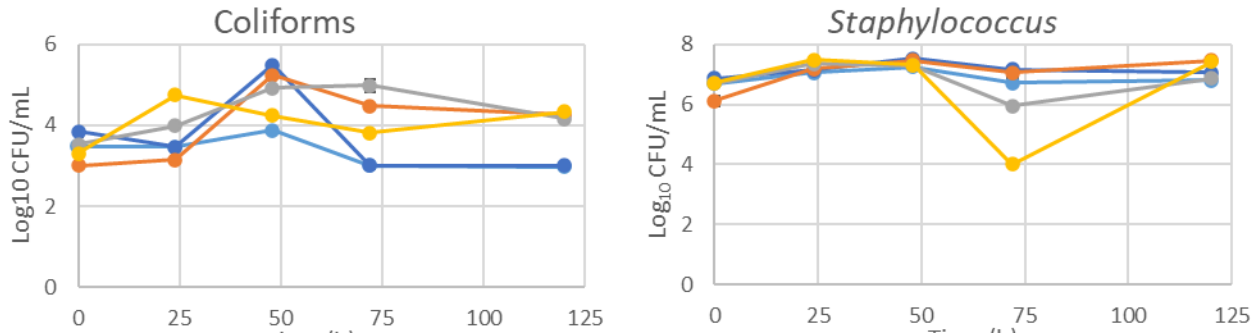

Figure 3. Bacterial counts for each group of organisms in RMS with varying percentage of relative humidity of the RMS, expressed as Log10 CFU/g dry matter. The moisture percentage tested were Control (light blue), 30% (dark blue), 60% (orange), 75% (grey) and 90% (yellow).

3.1.4. Conclusions and next steps

Further conclusions will be sequentially added once results from the characterization of the manure in vitro and the screening of products is finalized.

Pasteurization and Composting Reduce Pathogens: Both pasteurization and composting processes are effective in reducing coliforms, fungi, and yeast in recycled manure solids (RMS). However, these treatments have limited impact on reducing Staphylococci and Streptococci counts.

Dry Matter Content Is Critical: Clean RMS samples generally have similar dry matter percentages in our population (about 37.5–40%), while used RMS can vary more widely depending on farm management and climate. Maintaining dry matter above 32–34% is important to inhibit pathogen growth and ensure bedding hygiene.

Humidity and Temperature Affect Bacterial Growth: Lower humidity (0–30%) in RMS bedding prevents the proliferation of coliforms and other pathogens, while higher humidity (60–90%) increases bacterial growth. High temperature (65ºC) further reduces bacterial counts, but temperatures above 70ºC may be needed for more effective pathogen inactivation.

Management Recommendations: Proper management of RMS bedding—including regular maintenance, controlling humidity, and ensuring adequate pasteurization or composting—is essential to minimize pathogen load and maintain cow health.

3.1.5. References

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3.2. S/T2: Dietary interventions

3.2.1. Introduction

3.2.1.1. Background.

Livestock production systems are a major contributor to global greenhouse gas (GHG) emissions, accounting for approximately 14.5% of anthropogenic emissions worldwide [Gerber et al., 2013]. Among these emissions, nitrogen excretion and subsequent ammonia volatilization represent critical environmental challenges. Ammonia emissions contribute to air pollution, acidification, and eutrophication, while also serving as precursors for secondary particulate matter formation [Sutton et al., 2021]. Traditionally, mitigation strategies have focused on manure management post-excretion, such as storage and land application techniques [Montes et al., 2013]. However, these approaches address emissions only after nitrogen has been excreted, missing an opportunity to intervene earlier in the nutrient cycle.

Improving nitrogen utilization efficiency during digestion offers a proactive strategy to reduce nitrogen excretion at its source. By enhancing the conversion of dietary nitrogen into productive outputs—such as milk and muscle tissue—emissions can be partially reduced before manure management becomes necessary. This approach not only mitigates environmental impacts but also improves animal performance and farm profitability, creating a synergistic benefit for sustainability [Hristov et al., 2022].

3.2.1.2. Current Challenges.

Conventional livestock diets often include high levels of crude protein to ensure adequate amino acid supply. However, excessive dietary protein leads to inefficient nitrogen utilization, resulting in elevated nitrogen excretion and ammonia emissions [Patra & Yu, 2022]. Furthermore, the European Union (EU) livestock sector relies heavily on imported soybean meals as a primary protein source. This dependency raises concerns about supply chain resilience and environmental sustainability, as soybean imports are associated with significant CO_2-equivalent emissions due to land-use change and transportation [European Commission, 2023].

3.2.1.3. Policy Context.

The EU has recognized these challenges and introduced initiatives under the **Green Deal** and **Farm to Fork Strategy** aimed at reducing the environmental footprint of animal production. The **EU Protein Strategy (2023)** emphasizes reducing dependency on imported soybeans and promoting alternative protein sources, including locally grown crops and circular feed systems [European Commission, 2023]. Additionally, policy frameworks encourage lowering dietary protein levels to reduce nitrogen excretion and associated emissions, while maintaining animal health and productivity [European Commission, 2022]. These measures align with broader climate neutrality goals and reflect a shift toward integrated approaches that combine nutritional innovation with environmental stewardship.

3.2.1.4. Knowledge Gap.

Despite policy momentum and scientific recognition of the benefits of dietary interventions, practical strategies for improving nitrogen efficiency remain underexplored. Most research has focused on reducing crude protein levels, but this approach alone may compromise animal performance if not accompanied by targeted supplementation. There is a need for innovative solutions that maintain or enhance productivity while reducing nitrogen excretion. This includes exploring the role of specific nutrients—such as isoacids and cobalt—in optimizing rumen fermentation and microbial protein synthesis [Patra & Yu, 2022; Hristov et al., 2022].

3.2.1.5. The Dietary Interventions Initiative.

The *Dietary Interventions* initiative aims to address these gaps by developing and validating nutritional strategies that improve nitrogen utilization efficiency in livestock. The core concept is to enhance the conversion of dietary nitrogen into productive outputs, thereby reducing nitrogen excretion and ammonia emissions at the source. This proactive approach complements downstream manure management strategies and offers multiple benefits:

- **Environmental**: Lower nitrogen excretion reduces ammonia emissions and nitrate leaching.
- **Economics**: Improved feed efficiency enhances profitability.
- **Animal Welfare**: Optimized diets support health and performance.

A key sustainability dimension of this initiative is reducing reliance on imported soybean meals by improving the utilization of existing feed resources, including fiber. This aligns with EU objectives to decrease the CO_2-equivalent footprint of feed ingredients and promote regional feed autonomy [European Commission, 2023].

Increasing microbial synthesis and fiber digestibility in the rumen is a key aspect of improving ruminal efficiency. Supplementation of cobalt is one potential approach. Cobalt is required by specific

bacteria to synthesize vitamin B12 which, in turn, is a growth factor for fibrolytic bacteria (Spears, 2020). Satisfying this requirement increases both bacterial growth and fiber digestibility. Another such approach is the supplementation of branched-chain fatty acids or isoacids. Fibrolytic bacteria are unable to transport preformed branched-chain amino acids across their cellular membrane and must rely on cross-feeding from proteolytic bacteria to supply these carbon skeletons for the synthesis of branched-chain amino acids and long chain branched-chain fatty acids (Firkins et al., 2024). Supplemental isoacids may be more efficiently utilized by fibrolytic bacteria vs obtaining them via cross-feeding mechanisms. Both types of supplementations tend to be effective when applied to high forage diets. Increased fiber digestion in high-forage diets is commonly associated with greater production of methane (Parnian-Khajehdizaj et al., 2023). Being one of the greenhouse gases with the greatest global warming potential, an increase in methane production would negatively affect the sustainability of dairy production. However, it is also suggested that improving microbial anabolism can act as a sink for methane precursors, CO2 and H2, that result from increased fiber digestion (Yi et al., 2023). Moreover, cobalt is involved in propionate-producing pathways (Tiffany et al., 2006) which also act as an H2 sink. Thus, the objective of this study was to improve the understanding of the impact of two organic cobalt sources (Cobalt glucoheptonate – CoPro and (Cobalt source under development) CobaltD) and isoacid supplementation on the methane production during ruminal fermentation and evaluate potential synergy between the two.

The main intervention in this study centers on the use of isoacids to improve the efficiency of rumen-degradable protein. This strategy can help reduce relative ammonia emissions by enhancing microbial protein synthesis, which represents the most efficient protein source for ruminants. The protein sources selected for comparison reflect common practices within the EU and align with current policy priorities. In the EU, soybean meal—largely imported and associated with a higher carbon footprint—has a less favorable outlook compared with protein supplements derived from locally produced crops such as canola/rapeseed.

A substantial body of scientific literature already supports the role of isoacids as modulators of rumen fermentation (we have attached an excellent review by Dr. Firkins from Ohio State University). Additionally, this project has generated two new abstracts that have been submitted to scientific conferences, which we will be happy to share once they are accepted by their respective scientific committees.

3.2.1.6. Specific Nutrients: Isoacids and Cobalt.

Isoacids—branched-chain volatile fatty acids—play a critical role in rumen microbial metabolism, particularly in the synthesis of branched-chain amino acids. Supplementation with isoacids has been shown to enhance microbial protein synthesis and improve nitrogen retention in ruminants [Patra & Yu, 2022]. Similarly, cobalt is an essential trace mineral required for the synthesis of vitamin B12, which supports propionate metabolism and energy balance. Adequate cobalt supply can influence rumen fermentation patterns and nitrogen utilization efficiency [Hristov et al., 2022]. The initiative will evaluate the impact of these nutrients on nitrogen efficiency, fiber digestibility, and overall animal performance.

3.2.1.7. Significance.

By targeting nitrogen efficiency during digestion, the *Dietary Interventions* initiative offers an approach to emission mitigation. This strategy reduces the need for downstream interventions, aligns with EU sustainability policies, and delivers co-benefits for productivity and animal welfare. Moreover, by decreasing reliance on imported soybeans, the initiative contributes to reducing the

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carbon footprint of feed supply chains and supports regional feed autonomy. Collectively, these outcomes position dietary interventions as a cornerstone of sustainable livestock production.

3.2.2. Methodology

3.2.2.1. Experimental Design

The study will employ two complementary vitro fermentation systems: **continuous culture** and **batch culture**, designed to simulate rumen conditions under controlled laboratory environments. These systems allow for precise evaluation of dietary interventions on nitrogen utilization efficiency, fermentation dynamics, and methane emissions.

Since this WP2 initiative will be carried out in vitro, CECOAGRO has planned the purchase of raw materials in advance. For each experiment, all raw materials are analyzed for their nutritional composition, and new dietary formulations are prepared accordingly to account for natural variation in supplemental protein sources. The experimental design follows a Latin square arrangement, allowing us to systematically include a control treatment to assess the specific effects of isoacids and/or the protein source.

3.2.2.2. Continuous Culture Fermentation

Continuous culture systems will replicate the rumen environment under steady-state conditions, enabling accurate measurement of digestion efficiencies and microbial activity. The design will follow a **Latin square arrangement** using four fermenters to minimize variability and account for potential carryover effects. Each run will last **13 days**, including an adaptation period and a sampling phase.

Parameters assessed in continuous culture include:

- **Organic matter digestibility**
- **Fiber degradation** (Neutral Detergent Fiber [NDF] and Acid Detergent Fiber [ADF])
- **Microbial protein synthesis**
- **Volatile fatty acid (VFA) profiles** to evaluate fermentation patterns and energy supply.

These measurements will provide insights into nitrogen efficiency by monitoring nitrogen flow and retention within microbial biomass.

3.2.2.3. Batch Culture Fermentation

Batch culture fermentations will complement continuous culture studies by focusing on **methane emissions** associated with the same dietary interventions. A total of **18 vessels** will be used, with treatments replicated **2–3 times** to ensure statistical robustness. Each batch run will last **2 days**, allowing rapid screening of treatments for their impact on enteric methane production. Controls will be included in all experiments to establish baseline values for comparison.

Methane production during ruminal fermentation was studied through batch tests using the Gas Endeavour III (BPC Instruments, Sweden). The system was composed of 18 1-L glass vessels with airtight stoppers and mechanical stirring. The vessels were individually connected to CO_2 traps (NaOH ≥3M mixed with thymolphthalein as pH indicator) and to gas-measuring cells working by liquid displacement/buoyancy. Data of each vessel was recorded and visualized within BPC instruments software (Aurora™). The duration of the batch was set at 24 h after the inoculation. Each vessel was filled with 200 mL of inoculum collected from cannulated Holstein cows and 400 mL of buffer solution (prepared similarly to Roman-Garcia et al. (2021)). The diet was fed as 25 g DM per vessel and its composition was as follows (% DM basis): 35.2% corn silage, 22.9% grass silage, 17.6%

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fine-ground corn grain, 11.1% soybean meal, 6.88% dry beet pulp, 4.59% ground soybean hulls, 1.15% wheat straw, 0.43% urea, 0.19% mineral mix. An isoacid blend (IA) was fed at a 40g/kg DM ratio whereas both cobalt sources (CoPro and CobaltD) were fed at 1.0 ppm Co/kg DM.The treatments were formulated as follows: positive control (CON), isoacids (IA), cobalt glucoheptonate (COPRO), cobalt amino acid (COBALTD), isoacids mixed with cobalt glucoheptonate (COPRIA) and isoacids mixed with cobalt amino acid (COIA). All treatments were tested by quadruplicate, except for the positive control by duplicate.

CECOAGRO will use soybean meal and canola meal as the primary protein sources for comparison. Although ruminant diets include additional protein sources (forages, bypass proteins, etc.), these two ingredients represent the main supplementary protein sources in the EU based on their dry matter contribution. Therefore, they form the focus of the evaluation. Soybean and canola meals are the foundation of protein supplementation in ruminant diets. Isoacids are applied to correct the deficit in branched-chain amino acids that limits fiber digestion and microbial protein synthesis in the rumen. Enhancing the efficiency of protein-nitrogen digestion will allow the industry to formulate diets with lower overall protein levels without compromising farmer profitability or animal health, while achieving a net reduction in nitrogen-related emissions. This intervention is also aligned with new EU policies aimed at reducing total protein supplementation, especially in dairy cow diets.

The focus of this WP2 nutritional intervention is on ruminant diets. Although there is potential to extend this research to monogastric species such as poultry or swine, the funding currently allocated to CECOAGRO within the project does not cover the costs of evaluating the intervention beyond ruminants.

3.2.2.4. Dietary Strategies Tested

The initiative will evaluate essential nutrients and their interaction with different protein sources and fiber levels. Specific interventions include:

1. **Isoacids supplementation**: Branched-chain volatile fatty acids to enhance microbial protein synthesis and nitrogen retention.
2. **Cobalt as a mineral additive**: Required for vitamin B12 synthesis, influencing propionate metabolism and nitrogen utilization.
3. **Adjustments in protein levels and fiber utilization**: Lowering crude protein content while optimizing fiber digestibility to improve nitrogen efficiency and reduce excretion.
4. **Protein source comparison**: Canola meal (more available in the EU) versus soybean meal, as well as sustainable alternatives such as sorghum, grass silage, and corn silage.
5. **Determination of optimal feeding rates** for isoacids and cobalt to maximize efficiency without compromising animal health or productivity.

3.2.2.5. Species and Housing Systems.

The primary focus will be on ruminants, particularly dairy cattle, due to their significant contribution to nitrogen excretion and ammonia emissions. Comparative studies may include swine and poultry to assess cross-species applicability of dietary interventions, only contingent to the progression of the budget allowance.

3.2.2.6. Statistical Analysis.

Data will be analyzed using **mixed-effects models** to account for repeated measures and hierarchical structures. Treatment effects will be evaluated using ANOVA, with significance set at $p < 0.05$. Replicates will be clearly defined for each experimental unit to ensure statistical robustness.

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3.2.3. Results

Results from currently finished experiments will be posted here sequentially once the analysis of the data is finished. All treatments, except for isoacids alone (Figure 4a), showed greater methane production than the control (Figure 4b). This tendency suggested that fibre fermentation is indeed promoted by the inclusion of cobalt sources. The Cobalt [under develop] (COBALTD) appears to be more efficient at providing cobalt to the microbiome given the greater increase in gas production in comparison with the glucoheptonate (COPRO). Interestingly, the positive change in gas production is partially offset when isoacids are mixed with both cobalt sources (Figure 5), especially in the Cobalt [under develop] case (COIA). The inclusion of IA might indicate IA-promoted fibrolytic bacteria growth might serve as a sunk for CO2 and H2. Overall, the percentage change between control and all treatments slightly diminished overtime possibly because treatments affected fermentation kinetics, speeding up NDFd and subsequent gas production especially at the beginning of the test (3 h).

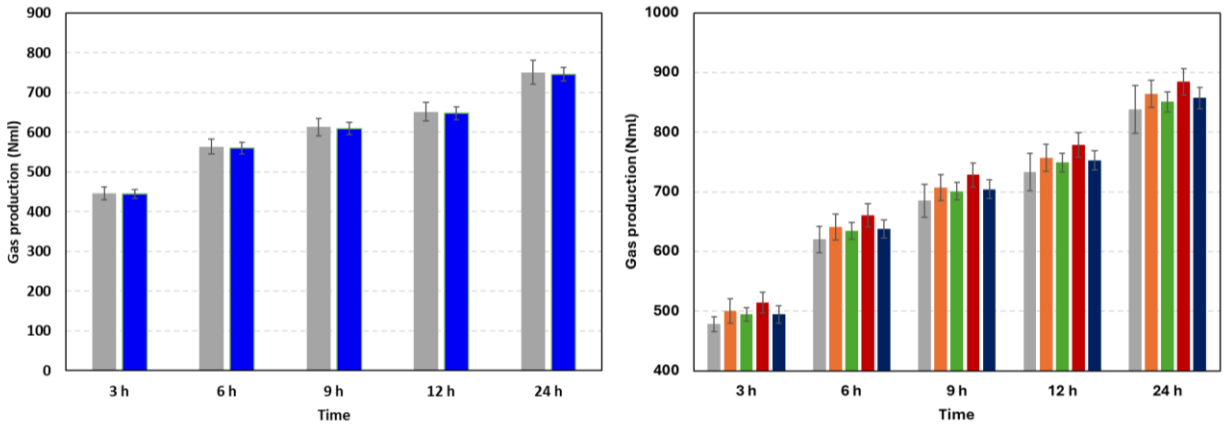

Figure 4. Cumulative methane production at different time points of the test: a) comparison between control (CON, grey) and isoacids alone (IF, blue); b) comparison between control (CON, grey) and the cobalt source without (COPRO, orange, COBALTD, red) and with isoacids (COPRIA, green, COIA, dark blue).

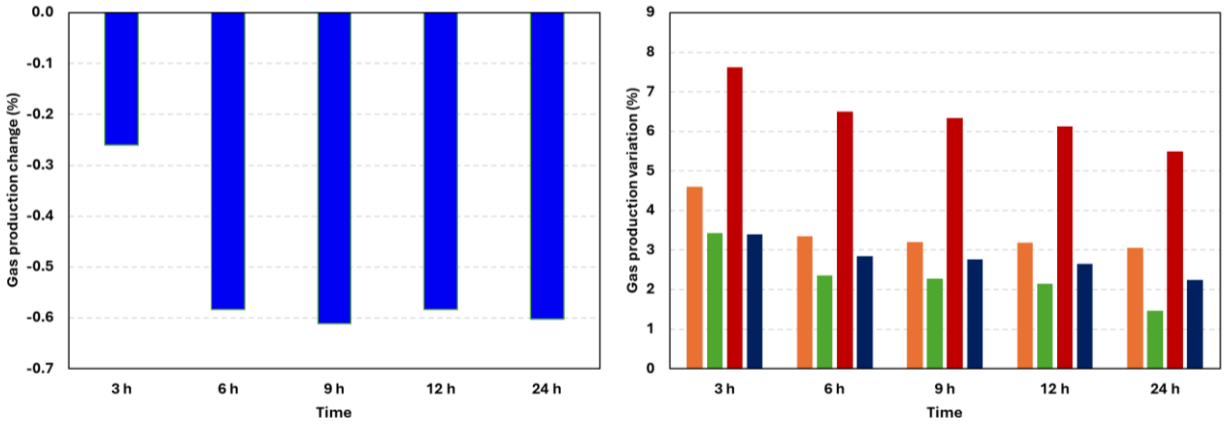

Figure 5. Methane production percentage change at different time points of the test: a) comparison between control (CON, grey) and isoacids alone (IF, blue); b) comparison between control (CON, grey) and the cobalt source without (COPRO, orange, COBALTD, red) and with isoacids (COPRIA, green, COIA, dark blue).

3.2.4. Conclusions and next steps

Based on methane yields, it was concluded that the cobalt Cobalt [under develop] is more efficient at promoting fiber fermentation than its glucoheptonate form, possibly due to greater bioavailability and/or microbial transmembrane transportation. Regardless of previous literature indicating increased fiber fermentation, isoacids supplementation does not affect methane production significantly, reducing concerns related to its sustainability impact. Supplementing isoacids together with cobalt, especially in the form of Cobalt [under develop], provides a synergistic opportunity to increase fiber degradation in the rumen and generate greater microbial protein while partially offsetting the increased methane yield. In fact, it is hypothesized that part of the excess H2 and CO2 produced with cobalt supplementation are then sunk into fibrolytic bacteria anabolism.

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4. GHG emissions reduction primary treatment strategies

4.1. S/T3: Biochar addition in Anaerobic Digestion (AD) processes

4.1.1. Introduction

Green House Gases (GHG) emission presents a prejudicial effect on climate change and public health. Normally, the focus is located over C02 emissions due to the increase quantity of these emissions for the human activity. However, other GHG's gases present a superior greenhouse effect as the methane, with 84 g CO2eq. The emission of methane of the manure is inseparable of the biodegradation of this material. However, these emissions can be captured although the anaerobic digestion system.

Biomethane production is one of the main renewable energy production systems, include in Repower EU plan (European Commission, 2022), for reduce the energy dependence of the exterior agents. Biomethane production is development through a biological process called Anaerobic Digestion (AD). AD is carried out by the microbiological degradation or organic matter to biogas, C02 and CH4, and a residual effluent called digestate.

DA systems show a Climate change mitigate effect due to the capacity to avoid GHG's emissions. CH4 and C02 production is isolate in the reactor and prevent the atmosphere emission. Furthermore, these gases can be purified and valorised to add-value-products, such energetical vectors. However, the medium-low efficiency of the process represents a disadvantage to the AD system expansion as a GHG's mitigate system. With the aim of upgrade, the AD efficiency is proposed the carbon-base material (CBM), addition. Several studies show a relationship between CBM addition and the increase of biogas production (Qiu et al., 2019; Khalid et al., 2021; Paritosh et al., 2020).

4.1.1.1. CBM production and characteristics

CBM can be produced for different technologies attend on process conditions and substrate materials. The CBM synthesis technology has present an exponential development during the last years to respond to waste production. It exists diverse strategies focus to the feedstock characteristics or the desirable obtained product. Main CBM production techniques defer to the operation, feedstock characteristics and obtained products (Table 2). To avoid the organic matter combustion and total degradation, every CBM synthesis operations is carries out in anaerobic conditions.

Table 2. CBM production technologies and her requirements.

Technology	Temperature and pressure	Feedstock characteristic	Products obtained
Pyrolysis	250-625ºC	Maximal moisture content: 80% Maximal ash content: 61%	Biochar, Bio-oil y Syngas
Torrefaction	200–300 °C	Maximal moisture content: 80% Maximal ash content: 61%	Biochar y Syngas
Gasification	750-900ºC	Maximal moisture content: 80% Maximal ash content: 61%	Biochar, ashes, Tar y Syngas

Hydrothermal carbonization	180-260ºC 1-5 MPa	Moisture: 50%-90%	Hydrochar, Bio-crude oil y Syngas
Hydrothermal liquefaction	180-400ºC 2-25 MPa	Maximal moisture content: 95% Maximal ash content: 57%	Hydrochar, Bio-crude oil y Syngas

The feedstock composition is another factor to take account that presets a significant influence over the CBM quality. On one hand, the feedstock determinate the chemical parameters of CBM, as ion concentration or surface functional groups. Micronutrients can be released to increase the development of microbial communities while the ion liberation produces a buffer effect. However, the feedstock shows a direct effect on the physical characteristics, as the hydraulic behaviour of CBM.

Operation Temperature act as a critical parameter to determinate the CBM properties, which affect directly to AD. At lower temperatures (200-400ºC), the O content at the CBM increase, while the content of C, H and O decrease in high temperature processes (600-1000ºC).

Less labile chemicals on the surface of the charcoal particles in high-temperature BC result in fewer microbial substrates for methanogenic archaea and fermentative bacteria. Overall, the results demonstrate that BC produced at temperatures lower than 700°C enhances methanogenesis (Xiao et al., 2021). In addition, CBM produced through high temperature processes show a negative effect over the methane production due to the production and liberation of inhibitory compounds (Das et al., 2021).

4.1.1.2. CBM applied in AD

One of the most studied uses of CBM is its use as an additive to the AD process. This use is due to the advantages of CBM to enhance the system stability and the biogas production thanks to the special and physicochemical CBM characteristics. These effects cover the physicochemical effects (ion exchange interactions, inhibitors adhesion, etc) and microbiology effect (enhancing the microbial populations diversity, promote the bacterial metabolism, etc). Furthermore, the AD allows the use of CBM as an additive thanks to its viability and technological maturity, adding value to it and providing a commercial outlet for this product.

Numerous studies have shown that adding biochar to digesters enhances microbial diversity and population, especially under suboptimal conditions. This improvement is attributed to biochar's properties, such as higher porosity and larger surface area, which provide a suitable habitat for microorganisms. Additionally, biochar enriches specific microbes as electrotrophic methanogens species, leading to increased methane production by facilitating electron transfer and syntrophic metabolism (Zhao et al., 2024). In addition, the CBM supplies micronutrients and trace elements to microorganism, enhancing the microbial growth due to the extra essential elements.

Increase the metabolic activity thought the boosting the functional enzyme and gene activity, promoting the secretion of extracellular polymeric substances and accelerating electron transfer rate (Zhao et al., 2024). Factors influencing this DIET include SSA and porosity, which increase with rising temperatures (700°C or 900°C) exhibits an enhanced DIET mechanism (Kundu et al., 2023) . Direct interspecies electron transfer (DIET) is the name gave to some metabolic pathways which occurs between different microbial species. These pathways can promote the methane production for the acetate degradation (acetotrophic) or the hydrogen reaction with the CO_2 (hydrogenotrophic).

CBM shows conductivity activity in aqueous solution, thanks to functional groups on the surface of CBM. This activity favoured the

The CBM addition have a direct effect over the AD inhibition by components as ammonia (NH_4) and Volatile Fatty Acids (VFA). VFA accumulation promotes the methane production inhibition due to the Ph decline with a toxic effect over the methanogenic bacterial. Equally, VFA accumulation acts as a warning signal inhibitors presence, which decrease the VFA consumption of the methanogenic bacteria and increase the VFA concentration in the medium. The additional alkalinity, promoted of the CBM addition, can reduce the VFA inhibition in overload episodes or inhibition scenarios (Kundu et al., 2023). Additionally, the NH4 inhibition's mitigation present a double mechanism: physicochemical interaction between CBM and NH_4, and biofilm formation inside CBM. Likewise, biofilm formation inside the CBM allows a physical separation between microorganism and NH4. Reduction of inhibitor-microorganism contact support the microbial growth and internal colonization of CBM. The internal colonization is carried out of diverse microbial species, including methanogenic *archaea* genera. Some methanogenic genera, well descripted, are *Methanosaeta* and *Methanosarcina*, present a differential colonization of CBM, adapting the growth to the structural characterises of CBM (Lü et al., 2016).

Finally, it is relevant the influence of the postproduction treatment of CBM over the enhancing of biogas production and process stability. Low particle size increases the microbial CBM colonization through the reduction of dead-zones inside of the material (Lü et al., 2016). This effect promotes the benefits of the CBM application and allows modify the ÀD performance.

4.1.2. Methodology

4.1.2.1. CBM source and characterization

The CBM selected to the AD additive with CBM was produced from the dry olive pomace pyrolysis (Carboliva S. L., Spain). The resulting biochar was characterized by pore size distribution using CO2 adsorption isotherms at 77 K, applying the BET model with a surface area and porosity analyser (Micromeritics Tristar II Plus). This equipment is able to determinate the material specific surface (SS), pore size (PS) and pore volume (PV) were characterised by N-absorption exothermic curves.

4.1.2.2. CBM dosage selection by BMP test

Previous of the optimal dosage selection, it was carrying out a bibliographic analysis to define the CBM dosage used in anaerobic digestion as additive. To this aim, it was development the revision and data normalization of the dosage used in scientific articles (n=125), adapting it to the unis used in AINIA (g/L). Therefore, was realised a pareto plot and a box plot to identify the most used dosage.

To determinate the optimal dosage to the AD assay, it has been defined different dosage to evaluate it although a BMP test of the substrate objective, raw pig manure. The BMP test was selected due to the large AINIA's experience in this test and the robustness of the assay to additive evaluate.

Biomethanization tests (BMP) will be carried out at the pilot plants available at AINIA, following the procedures and methodology developed by AINIA in accordance with the standard issued by the Association of German Engineers: VDI 4630:2016-11, titled "Fermentation of Organic Materials – Characterization of the Substrate, Sampling, Collection of Material Data, Fermentation Tests."

The test allows for the evaluation of the maximum biogas production potential of the samples, the possible occurrence of fermentation process inhibition (qualitatively), and the degradation rate of the

substrate (also qualitatively). However, this test does not provide information regarding the organic loading limit or the stability of the process under continuous operation conditions.

Regarding the inoculum to be used in the BMP tests, it will consist of digested material sourced from the anaerobic digester at the municipal wastewater treatment plant (WWTP) in Paterna (Valencia). Upon request, it is possible to use an inoculum provided by the company for these tests. The cost of tests using company-supplied inoculum will be assessed by the project's technical lead.

The biogas volume will be continuously measured using volumetric counters. Subsequently, a chemical analysis of the biogas composition will be performed to determine the methane (CH_4) yield of the substrate under study. In the 2-liter digesters, gas composition is analysed using a specific infrared sensor analyser (AWITE Multitec) (Figure 6A). In the AMPTS III system, methane production is determined by the volumetric difference (under standard conditions) between the total gas produced and the gas remaining after passing through a 3M NaOH solution (for CO_2 absorption) (Figure 6B).

A)

B)

Figure 6. BMP systems of AINIA. A) AINIA's 2L batch digesters and B) AMPTS.

4.1.2.3. Semi-continuous assay

Semi-continuous anaerobic digestion tests will be carried out under standard conditions (pilot scale, completely mixed configuration, once-daily feeding) in 2 10L-CSTR reactors (Figure 7), operated under mesophilic conditions (38ºC). The feedstock mix is constituted by raw pig manure in both reactors. The reactor A will be the Control reactor, while the reactor B will be the Probe reactor. The reactor B will be operated with the CBM addition to look the differences in biogas production, biogas quality and digestate quality between them.

Figure 7. 10L-CSTR used by AINIA in Semi-continuous AD assays.

The current technology readiness level (TRL) for anaerobic digestion is 9, given its industrial-scale application. However, the incorporation of biochar into digesters remains at a lower TRL, between 4 and 5. This distinction is particularly relevant when considering additional process aspects, such as the potential for GHG reduction compared with conventional digestion systems.

This test will evaluate key aspects of the anaerobic digestion process, such as biogas volume (produced per ton of fresh matter introduced into the digester; per unit volume of the digester per day), biogas quality (composition in CH_4, CO_2, and H_2S), organic matter removal efficiency, and digestate quality, under semi-continuous feeding conditions (once daily). Furthermore, this test allows for the definition of optimal operating conditions for the process, such as Hydraulic Retention Time (HRT) or Organic Loading Rate (OLR), based on a feed mixture agreed upon with the company.

The initial inoculum will be provided either by AINIA and it will consist of digested material sourced from the anaerobic digester at the municipal wastewater treatment plant (WWTP) in Paterna (Valencia). The experimental procedure defined by AINIA includes a gradual increase in feeding until the appropriate OLR is reached, while maintaining the biological stability of the system. The semi-continuous anaerobic digestion test is expected to last for approximately three months in order to achieve steady-state conditions in the biological process. This extended start-up period is recommended based on the complexity of the residues comprising the feed mixture. Once steady state is reached, it will be maintained for two HRTs.

4.1.2.4. GHG measurement protocol

Anaerobic digestion is widely used to treat livestock manure and organic waste, producing biogas and a nutrient-rich digestate. After digestion, the digestate is typically stored before land application, during which greenhouse gas (GHG) emissions, particularly methane (CH_4), can occur. These emissions significantly influence the overall environmental impact of the treatment process.

The reduction of emissions during anaerobic digestion (AD) processes will be determined by comparison with untreated manure and with conventional AD systems without additives. Relative to raw manure, lower emissions are expected after the AD process due to the stabilization of organic matter during digestion. When compared with conventional digestate, a decrease in emissions should be achieved under identical operational conditions (OLR and HRT), as a result of increased process efficiency and nitrogen adsorption by the additives.

In this context, accurately estimating CH_4 emissions from stored digestate is essential for evaluating the climate performance of anaerobic digestion systems. This report presents an adaptation of the empirical model developed by Baral et al. (2018) to simulate CH_4 emissions from digestate produced in a semi-continuous anaerobic reactor and subsequently stored. The model integrates digestate characteristics, temperature dynamics, and degradation kinetics to predict methane release during storage.

This report outlines the adaptation of the empirical methane (CH_4) emission model described by Baral et al. (2018) to estimate CH_4 emissions from digestate stored after treatment in a semi-continuous anaerobic reactor. The goal is to simulate CH_4 emissions during storage based on digestate characteristics and environmental conditions.

The anaerobic digester used for this study operates under semi-continuous feeding conditions and is designed to achieve biological stability. A gas outlet will be installed at the top of the reactor, connected to a flowmeter to measure the biogas production rate. Downstream of the flowmeter, the biogas will be collected in a gas sampling bag. Along this line, a sampling port will be included to allow for periodic measurement of methane (CH_4) concentration in the biogas.

- Digestate characterization- To apply the model, it is essential to characterize the digestate exiting the anaerobic reactor. Key parameters include:
  - Total Volatile Solids (VS)
  - Degradable Volatile Solids (VSd)
  - Non-degradable Volatile Solids (VSnd)
  - Total Organic Carbon (TOC)
  - Ammoniacal Nitrogen (NH_4^+)
  - VSd: Based on methane (CH_4) production data from the reactor, it is possible to estimate the degree of volatile solids (VS) degradation achieved during digestion and to assess the remaining VS fraction that may still contribute to methane emissions during subsequent storage.
- Model Equations- Methane emission rate (F_CH_4) is calculated using the Arrhenius equation:

$$F\_CH_4 = (VSd + 0.01 \times VSnd) \times A \times \exp(-Ea / (R \times T))$$

  - Where:
    - A: Pre-exponential factor (g CH_4 kg^{-1} VS h^{-1})
    - Ea: Activation energy (kJ mol^{-1})
    - R: Gas constant (8.314 J mol^{-1} K^{-1})
    - T: Temperature in Kelvin
- Temperature Handling-Temperature is a critical driver of CH_4 emissions. For storage simulations, use:
  - Daily or weekly average ambient temperature
  - A moving average (e.g., 5-day) to smooth fluctuations
  - If possible, install temperature sensors in the storage tank to improve accuracy.
- Cumulative Emission Calculation- Daily CH_4 emissions are integrated over the storage period:

$$CH_4\_total = \Sigma\, F\_CH_4(t) \times \Delta t$$

  - Where Δt is the time step (e.g., 1 day). This provides total CH_4 emissions per unit of VS or per volume of digestate.
- Experimental Validation:
  - To validate the model:
  - Measure CH_4 emissions from the storage tank using gas sampling (e.g., Tedlar bags or sensors)
  - Compare measured emissions with model predictions
  - Adjust parameters A and Ea if necessary to improve fit
- Recommendations for Implementation:
  - Characterize digestate thoroughly before storage.
  - Monitor temperature continuously or use reliable estimates.
  - Apply the Arrhenius-based model with appropriate parameters.
  - Validate with experimental data and refine model inputs.

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- Use the model to simulate different storage scenarios and mitigation strategies.

4.1.3. Results

4.1.3.1. CBM source and characterization

The N-absorption assay allows draw the exothermic absorption curve (Figure 8) to determinate the SS and de PS/PV of the material, together with its characterisation. This assay defined the SS in 0,6257 ± 0,1650 m²/g. This SS is lower than other CBM used in AD, which have values between 18-200 m²/g (Qin et al., 2023; Ortiz et al., 2025). Therefore, it can be defined as a Low Surface Areas CBM (less than 250 m^2/g), adequate for combustion or soil restoration applications. Otherwise, it was determinate the PS and the PV in 88 Å and 0.00139 cm^3/g, respectively. This size is lower that the PS and the PV of used biochar as an AD additive (Ortiz et al., 2025). The PS and the PV have different implications over the AD, due to the microbial capacity to colonize these structures. The diversity of pore size has a positive effect over AD, providing support structures for diverse microorganism (He et al., 2024). As result of this characterization, is possible to recommend the CBM modification as a method to improve the SS and the additive AD performance.

Parameter	Value
Moisture content	8 - 12 %
Density	500 - 600 Kg/m3
Ash content	20 - 25 %
Organic matter content	15 - 20 %
Fix carbon content	55 - 60 % d. m.
Heat power	5.500 - 6.000 Kcal/Kilo

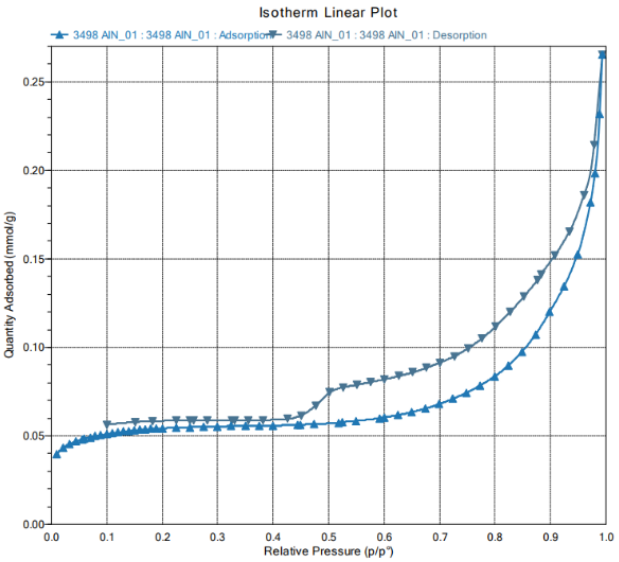

Figure 8. Physicochemical characteristics (A) and isotherm linear plot of the N-absorption (B) of selected CBM.

4.1.3.2. CBM dosage selection by BMP test

To obtain the optimal CBM dosage, AINIA will realise BMP test of CBM raw and modified in 3 different dosages (high, medium and low. To determinate these levels, was realised a Pareto plot and a box plot (Figure 9). This Figure shows most values between g/L. This determination allows define the CBM dosage in 5 g/L (high), 10 g/L (medium) and 15 g/L (low) in work volume of BMP tests.

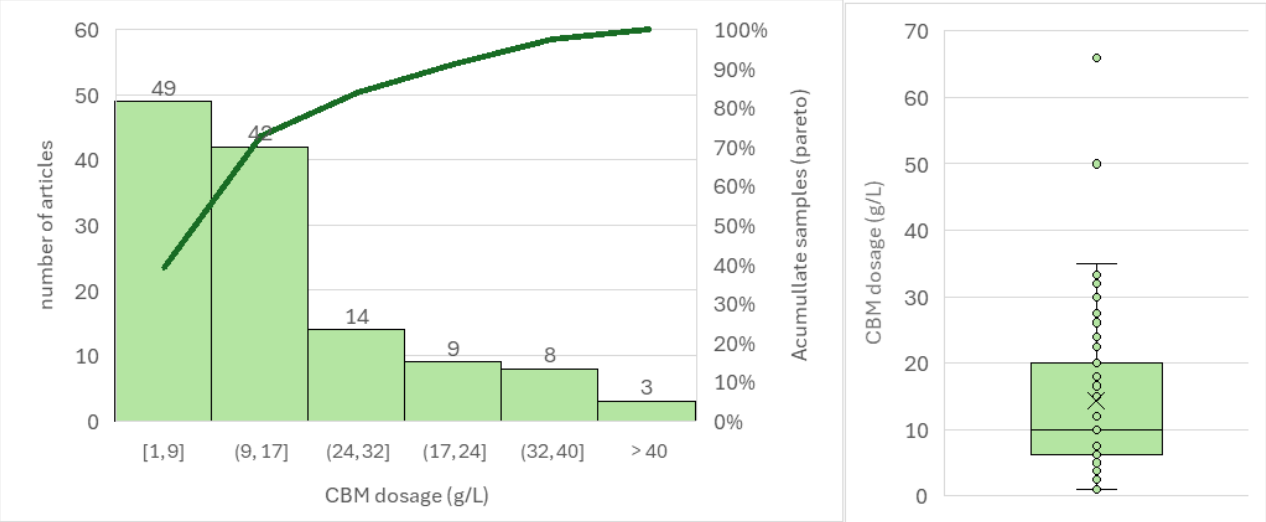

Figure 9. Distribution of CBM dosages used in bibliography in the application over AD processes. A) Pareto plot and B) Box plot.

4.1.4. Conclusions and next steps

The actual advance has conditioned the obtained conclusions of this first stage of the project. Actually, it's defined the objective dosages and the methodology to CBM addition evaluate over AD, as well as the methodology of GHG emissions measure. The nest steps and the timeline is in the Table 3.

Table 3. Timeline for the CBM addition assays in the AD for the GHG emissions reduction.

Next steps	2026							
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
BMP test to CBM dosages evaluation								
Semi-continuous assays								
GHG emissions from the digestate.								

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4.2. S/T4: Zeolite addition in Anaerobic Digestion (AD) processes

4.2.1. Introduction

As in the case of the methane in the ST3/Biochar, it exists other gaseous emissions with impact over the environment as the ammonia (NH_3). Ammonia is commonly released as a byproduct of agricultural and industrial activities. Typical anthropogenic sources include gaseous emissions from decomposing agricultural slurry and fertilizer production facilities. In addition, natural sources of ammonia emissions have been identified, such as the burning coal mines in Jharia (India), the alkaline waters of Lake Natron (Tanzania), and the guano deposits found in seabird colonies.

Once released into the atmosphere, gaseous ammonia can react with other airborne pollutants to form fine particulate matter composed of ammonium salts. These particles pose a risk to human health, particularly by impairing respiratory function. Furthermore, ammonia deposition can alter the chemical composition of soils, potentially affecting their fertility and ecological balance.

To reduce GHG emissions and other gaseous contaminants, it is proposed the use of zeolite additive AD. This combination of technologies allows the capture and optimization of methane emissions, along with the reduction of ammonia emissions captured by zeolites. To describe both mechanisms, the zeolite absorption and the AD improvement will be developed in the next sections.

4.2.1.1. Zeolite absorption of ammonia

The absorption of pollutants by materials used in water treatment processes typically follows an asymptotic kinetic behaviour when conducted under batch conditions. This characteristic enables the determination of the material's adsorption capacity and, consequently, facilitates the definition of its kinetic profile and optimal dosage for system design.

Natural zeolites offer the advantage of being more cost-effective due to their availability in nature. These minerals are porous aluminosilicates with a moderate adsorption capacity compared to other materials. For instance, using synthetic water containing 70 mg $N–NH_4^+$/L, Godifredo et al. (2023) reported an adsorption capacity of 9.44 mg $N–NH_4^+$/g with clinoptilolite. Similarly, Millar et al. (2016) observed an adsorption of 10.40 mg $N–NH_4^+$/g using bentonite with an influent concentration of 311 mg $N–NH_4^+$/L.

Although the adsorption rate of zeolites is relatively fast, it is not as high as that of other materials. Isotherm experiments indicate that at least three hours are required to reach 90% of their maximum adsorption capacity (Alshameri et al., 2014; Guaya et al., 2015; Yin & Kong, 2014). While the cation affinity order varies depending on the specific type of zeolite, most exhibit a strong preference for monovalent cations such as ammonium, followed by divalent and trivalent cations. This characteristic is important to define possible interactions ion-zeolite and design elution treatments. For example, the affinity order for clinoptilolite is as follows:

Cs+ > NH4 + > Pb2+ > K+ > Na+ > Ca2+ > Mg2+ > Ba2+ > Cu2+ > Zn2+

Zeolites remove ammonium from aqueous media through a cation exchange process, which occurs in three consecutive stages between the working matrix and the adsorbent solid. First, the cation must transition from the liquid phase to the liquid–solid interface. Once at the interface, the cation migrates to the surface of the solid and subsequently diffuses through the zeolite's porous structure until it reaches active exchange sites (Alshameri et al., 2014). These active sites are specific regions where cation exchange occurs. In the process described, sodium is typically exchanged for ammonium, although the specific cations involved depend on both the intended application and the material used.

4.2.1.2. Zeolite effect over AD

During the AD process, the organic nitrogen is degraded to ammonia by the protein assimilation processes. While low concentrations of ammonia may enhance the AD process, elevated levels can lead to system failure (Li et al., 2023; O'Connor et al., 2023). The high ammonia concentration is typically found in the AD of feedstock mixture composed with high proportion of manure, slaughterhouse residues or municipal solid waste (Yang et al., 2025).

Total ammonia nitrogen (TAN) in AD systems exists primarily in two forms: ammonium ions (NH_4^+) and free ammonia (NH_3, FAN), both of which can contribute to process inhibition either directly or indirectly (Lendormi et al., 2022; Mlinar et al., 2022). The relationship between these two forms is typically described by equilibrium equations dependent on pH and temperature (Xiao et al., 2022a). Free ammonia is generally considered the principal inhibitory agent due to its hydrophobic nature, which allows it to passively diffuse across microbial cell membranes. This diffusion can disrupt intracellular proton balance and induce potassium deficiency (Shi et al., 2017).

Once inside microbial cells, FAN binds with extracellular protons (H^+) and is converted to NH_4^+, altering intracellular pH. To restore proton homeostasis, cells actively expel potassium ions via energy-dependent membrane pumps, increasing the metabolic energy demand and impairing specific enzymatic functions (Mlinar et al., 2022; Peng et al., 2023a).

Reported inhibitory thresholds for TAN range from 3.4 to 5.77 g/L, with corresponding reductions in methane yield between 39% and 100% (Li et al., 2023). These variations are attributed to differences in operational parameters such as temperature, reactor configuration, and the composition of microbial communities within each system.

To mitigate the ammonia inhibition. It had been studied the addition of ammonia absorbents as zeolites. In diverse studies, the zeolite addition is related with the reduction of the ammonia inhibition and the enhancement of biogas generation (Tang et al., 2023; Ruiz-Bastidas et al., 2024). Consequently, the introduction of zeolite in AD process could be an improvement strategy to reduce the methane emissions and the ammonia emissions during the manure management chain.

4.2.2. Methodology

4.2.2.1. Zeolite effect over AD

The effect of zeolite on AD has been measured using the same protocol used by S/T3 Biochar for the BMP test and the semi-continuous test. The zeolite selected to this study is a natural zeolite apported by SEPIOL S.A. (Miner S.A. Group, Spain). The zeolite dosages evaluated was 0 (Dosage 0), 1 (Dosage 1) and 2 g/L (Dosage 2) and 4 g/L (Dosage 3) in BMP test. In addition, ammonia measures were realised before and after BMP test by volumetry test. The assays were carried out over raw pig manure directly (ND) and doped (D) by urea over AD inhibitory concentration (>4.000 mg N/kg) (Table 4).

Table 4. Physicochemical characterization of AD substrates to BMP tests.

Parameters	Units	Inoculum	SD	D
Total solids (TS)	%	2,4	9,93	9,93
Volatile solids (SV)	% TS	62,90	65,02	65,02
Total ammonia nitrogen (TAN)	mg N/kg	789	2.427	4.282

As with S/T 3, the current technology readiness level (TRL) for anaerobic digestion is 9, given its widespread deployment at industrial scale. However, the incorporation of zeolites into digesters remains at a lower TRL, between 4 and 5. This is particularly relevant when considering additional process aspects, such as the potential for GHG reduction compared with conventional digestion systems.

4.2.2.2. GHG measurement protocol

Ammonia volatilization was determined using an adaptation of the method described by van der Stelt et al. (2007). The experimental setup (Figure 10) consisted of a 1.3 L airtight Kilner jar containing 120 mL of digestate (12 mm depth) and a structure made of 1 mm gauge galvanized wire, which held a trap with 10 mL of 1 molar H_2SO_4. The volatilized ammonia (NH_3) was captured as ammoniacal nitrogen (NH_4-N) in the sulfuric acid. The effective headspace volume of the jar was 1 L.

The ammonium concentration in the digestate was measured at the start of the experiment (time zero). The jar was then incubated at 25 °C, and the concentration of trapped ammonia was measured over time: at 24 h, 48 h, 96 h, 192 h, and 384 h. At each sampling time, the Kilner jar was opened, the trap was replaced with a new vessel, and the jar was resealed (van der Stelt et al., 2007).

The quality of the digestate used was only measured at the beginning and end of the experiment to minimize volume changes. Water evaporation from the digestate was negligible because the container was sealed.

To facilitate analysis, the sulfuric acid solution was diluted with deionized water, and the pH was adjusted above 4 to achieve a concentration within the effective range of the test cells. Ammonium concentrations in the adjusted solution were determined colorimetrically (as NH_4-N) using the indophenol blue method (e.g., Harwood and Huyser, 1970), with Merck® test cells in a Spectroquant NOVA60 photometer (Merck KGaA, Darmstadt, Germany). The ammonium test cells had a range of 2–80 mg NH_4-N/L.

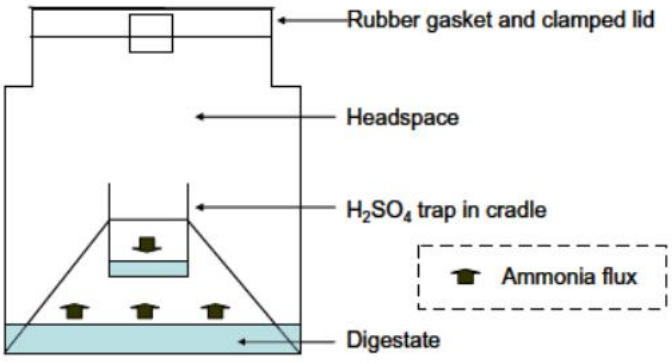

Figure 10. Schematic representation of ammonia stripping to air ammonia emissions quantification.

Stripping technology, traditionally used for the recovery of ammoniacal nitrogen, can also be adapted as a tool to evaluate the potential for ammonia volatilisation from digestate under controlled conditions. This approach is particularly relevant for assessing environmental risks associated with digestate storage and land application, as ammonia emissions contribute to air pollution and nitrogen loss.

In this study, the stripping setup is not intended to recover ammonium for reuse, but rather to simulate and quantify the amount of ammonia that could be emitted from the liquid fraction of digestate during storage. The methodology is based on the adaptation of the protocol described by van der Stelt et al. (2007) and is aligned with the mass transfer model proposed by Whelan et al. (2020), which characterizes ammonia volatilisation from anaerobic digestate.

Description of the Stripping Setup

A flow diagram is presented in Figure 11, numerically detailing the different components of the stripping setup and their operation. Each part is described below:

- Entry to the thermostatic bath: The bath is set to the desired temperature for the stripping test. A three-neck ground glass flask is placed in the bath and maintained at the set temperature during the test.
- Digestate input: The liquid fraction of the digestate is introduced into the three-neck flask. To promote ammonia release, the pH of the digestate is increased beforehand by adding bases such as caustic soda, thereby increasing the proportion of ammonia in gaseous form.
- Compressed air input: Compressed air is introduced into the process and regulated via a flowmeter.
- Air heating section: This section includes a heating element and hot water recirculation. Air is circulated through it for heating before being introduced into the three-neck flask.
- Heated packed column: Heated via a jacket through which hot water from the thermostatic bath circulates. Inside, the digestate and air (both preheated) flow in counter current. In water, ammonium is in chemical equilibrium with ammonia, depending on pH and temperature. The air moves in the opposite direction to the liquid, creating a concentration gradient that facilitates the transfer of ammonia from liquid to gas.
- Bubblers with hydrogen sulphide acid: These contain a solution of hydrogen sulphide acid at a specific concentration. Air containing ammonia from the packed column is bubbled through this solution via a submerged tube. As the air bubbles pass through the acid, gaseous ammonia dissolves and neutralizes, forming ammonium sulphate in solution.
- Hot water pumping: Hot water from the thermostatic bath is pumped to the jacket surrounding the packed column for heating.
- Digestate recirculation: A peristaltic pump circulates a specific amount of digestate from the three-neck flask to the top of the packed column. A flowmeter is used to regulate the recirculation flow rate.

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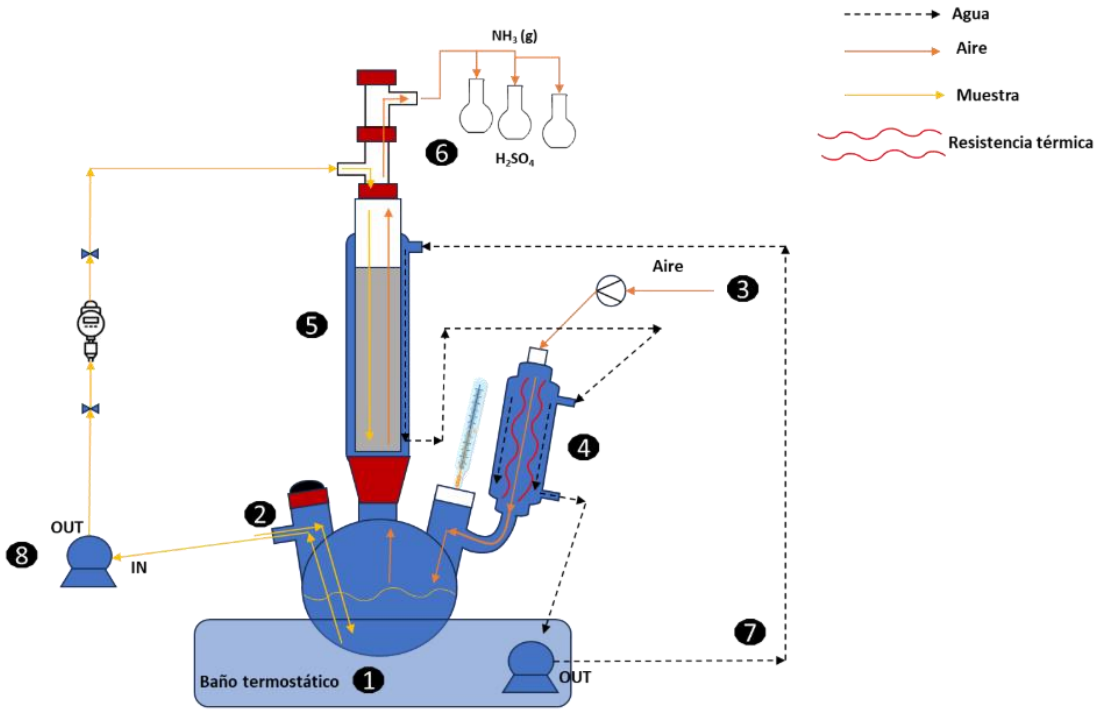

Figure 11. Flow diagram of the stripping setup.

Additionally, the initial concentration of ammoniacal nitrogen ($N\text{-}NH_4$) in the digestate will be measured at the beginning of the test, and the final concentration will be determined at the end of the experiment. By maintaining fixed operating conditions, the evolution of ammonium in the acidic solution (trapped ammonium) will be monitored over time (Figure 12).

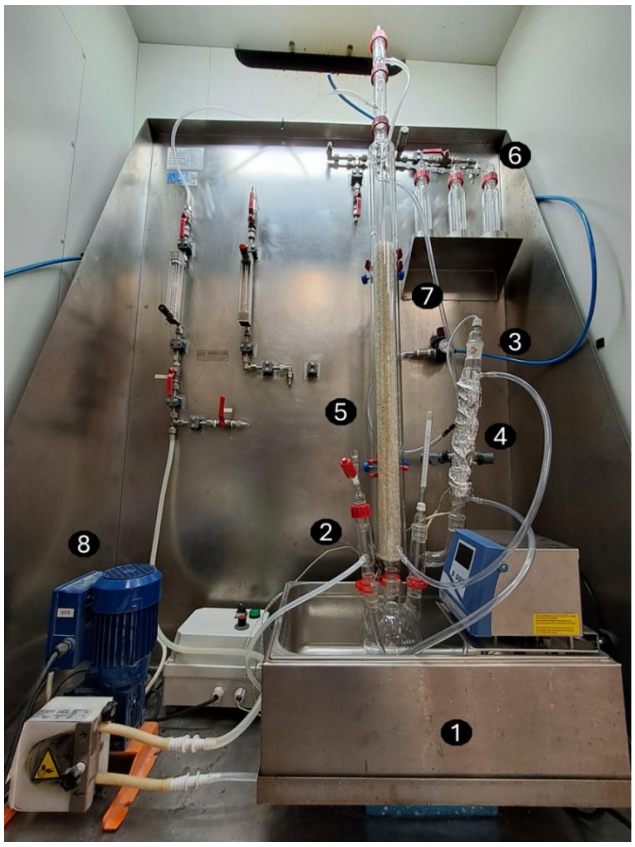

Figure 12. General view of the stripping setup in the pilot plant.

Mathematical Model to Estimate Ammonia Volatilization from Digestate

This dynamic model stimulates the emission of gaseous ammonia (NH_3) from stored digestate, considering chemical equilibrium between NH_4^+ and NH_3, mass transfer between phases, and NH_3 capture in an acid trap. It is based on the work of Whelan et al. (2020) and adapted from the experimental protocol by van der Stelt et al. (2007).

1. Initial Conditions and Unit Conversion: The model starts with a known concentration of total ammoniacal nitrogen in the digestate, primarily in the form of ammonium ion (NH_4^+):

   $$C\_NH4^+ = 5625 \text{ mg N/L}$$

   This is converted to molar concentration:

   $$C\_NH4 = C\_NH4^+ / MW$$

   Where MW is the molar mass of NH_4^+ (≈ 18 g/mol).

2. Chemical Equilibrium $NH_4^+ \rightleftharpoons NH_3$: An instantaneous and complete thermodynamic equilibrium is assumed between NH_4^+ and NH_3:

   $$C\_NH3 = C\_NH4 \times f\_NH3/NH4$$

   The fraction of NH_4^+ present as free NH_3 depends on the pH:

   $$f\_NH3/NH4 = 1 / (1 + 10\text{^} (pKa - pH))$$

   With pKa = 9.24 and pH = 8.3, a significant portion of NH_3 is in gaseous form, favoring volatilization.

3. NH_3 Transfer from Liquid to Air

The flux of NH_3 from the liquid to the headspace is calculated as:

$$J\_NH3 = A \times v\_aw' \times (C\_NH3liq - C\_NH3air)$$

It is assumed that $v\_a = 100 \times v\_w$, reflecting faster transport in the gas phase.

4. Temporal Evolution of NH_4^+ Mass:The mass of NH_4^+ in the liquid decreases due to volatilization:

$$dM\_NH4/dt = -J\_NH3$$

In discrete form:

$$M\_NH4(t) = M\_NH4(t-1) - \Delta t \times J\_NH3$$

5. Mass Balance in the Air:
The mass of NH_3 in the air increases due to volatilization and decreases due to capture in the trap:

$$dM\_NH3/dt = J\_NH3 - JTRAP$$

6. NH_3 Capture in the Acid Trap:
The transfer of NH_3 to the trap depends on its concentration in the air and the contact area:

$$JTRAP = kTRAP \times CNH3air \times Atrap$$

7. Updating Masses and Concentrations
NH_3 mass in the air:

$$M\_NH3(t) = M\_NH3(t-1) + \Delta t \times J\_NH3 - \Delta t \times JTRAP$$

NH_3 concentration in the air:

$$C\_NH3(t) = M\_NH3(t) / V\_a$$

NH_3 mass accumulated in the trap:

$$MTRAP(t) = MTRAP(t-1) + \Delta t \times JTRAP$$

NH_3 concentration in the trap:

$$CTRAP(t)=MTRAP(t)Vtrap$$

8. Physical-Chemical Parameters

$$va = 100 \times vw \text{ (TGD, 2003; Mackay, 2001)}$$

KAW = 0.00071 (Henry's law coefficient = 1.76 Pa·m³/mol at 25 °C, Sander, 1999)

Digestate pH = 8.3

9. Model Capabilities

Simulating NH_3 volatilization from digestate under controlled conditions.

Evaluating the impact of pH, temperature, interface area, and trap efficiency.

Estimating total NH_3 emissions during storage or treatment.

4.2.3. Results

4.2.3.1. Zeolite effect over AD

The BMP test results are showed in the

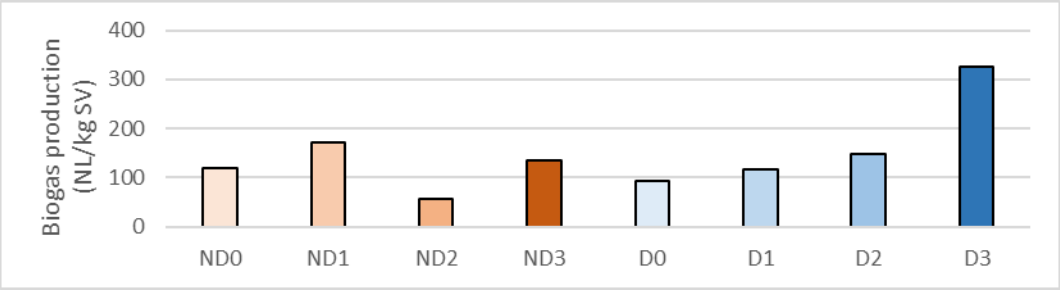

Figure 13, where it is observed the kinetical curves of biogas production throughout the assay. In this figure, it can be observed the improvement of biogas production between the Control samples (SD0 and D0) and the zeolite addition samples. On the right of the Figure 13, we can see the effect of zeolite addition over raw manure samples, where we can see a bigger biogas production in SD1 (172 NL/kg SV) and SD3 (135 NL/kg SV) samples than in the control SD0 (120 NL/kg SV). The SD2 sample shows a low biogas production during the start of the assay, reason for what it is necessary to refute this data. On the left of the Figure, we can see the effect of zeolite addition over doped raw manure samples, where we can see a bigger biogas production in D3 (325 NL/kg SV), D2 (148 NL/kg SV) and D1 (117 NL/kg SV) samples than in the control D0 (94 NL/kg SV). However, the several D3 production must be verified in subsequent tests and in the semi-continuous assay.

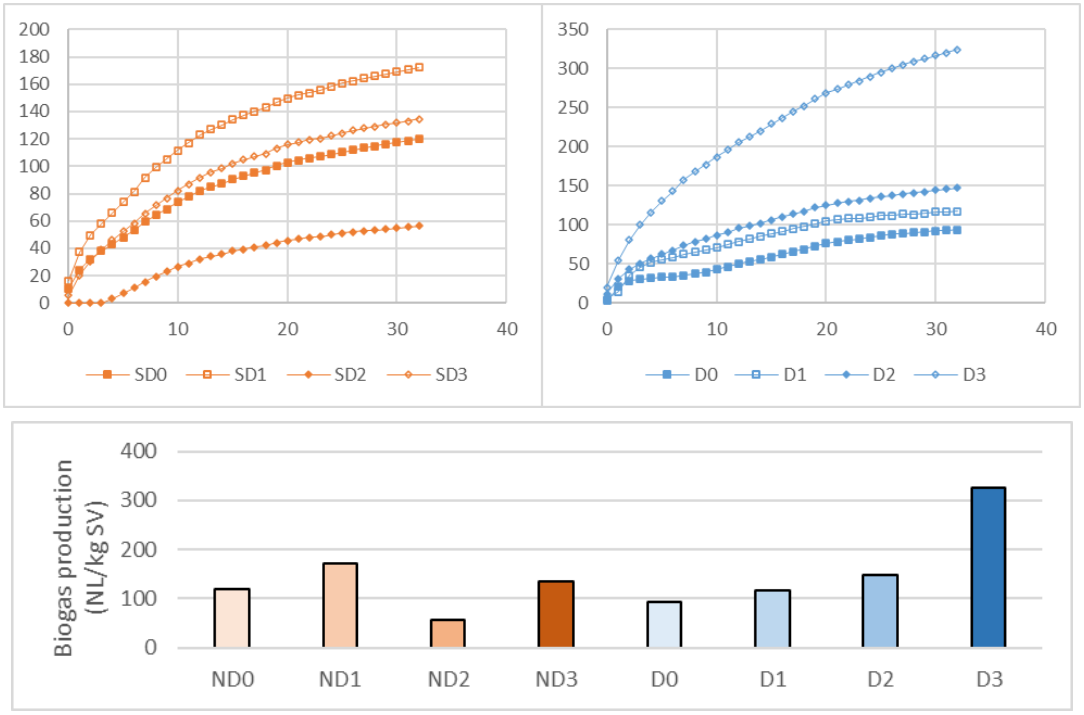

Figure 13. BMP test results of samples of raw manure (right), manure doped with urea (left) and biochemical methane potential value (bellow).

In the evaluation between the SD and D samples, the figure shows the ammonia inhibition between the SD0 sample (120 NL/kg SV) and the D0 sample (94 NL/kg SV), with a slow kinetic characteristic of microbial metabolism inhibition. Moreover, the high production of D zeolite samples over the ND zeolite samples can be explained due to the achievement of adequate zeolite absorption concentrations, mitigating ammonia inhibition (Liu et al., 2024).

Therefore, the degradation of organic matter by microorganisms promotes the ammonia liberation, with a direct relationship with the removal COD. This factor can cause an increase of the ammonia concentration in the samples of higher rates of organic matter conversion (Bardi et al. 2025). In the Figure 14, is observed the increase of TAN concentration (mg N/kg) during the BMP test of the samples in comparation with the initial concentration of the two substrates (NDi and Di) and the inoculum concentration after the assay. The increasement of TAN concentration after the BMP test is around the 20% of the initial TAN concentration of the studied samples. In the case of the doped samples (D), the increase of TAN concentration is more variable than in the no doped samples (ND). This aspect is related to the uneven degradation of the doped samples observed in the degradation kinetics of BMP test.

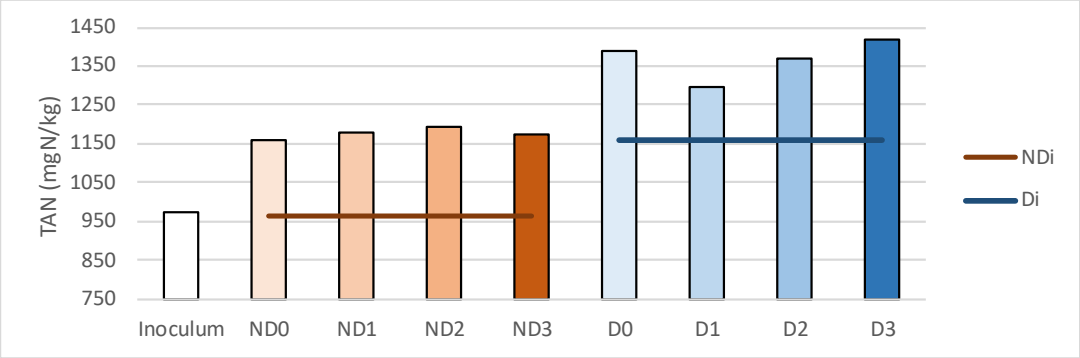

Figure 14. Concentration of TAN before (SDi and Di) and after the BMP test (t=31 days).

4.2.4. Conclusions and next steps

Based on the preliminary results, has been possible to select the 2 and 3 dosages to the Semi-continuous assay. In the next stage of the project, the BMP preliminary results will be validated to ensure the optimal dosage for the semi-continuous assay. The next steps and the timeline of the operative implemented in S/T4 Zeolites is defined in Table 5.

Table 5. Timeline for the Zeolite addition assays in the AD for the GHG emissions reduction.

Next steps	2025		2026			
	Nov	Dec	Jan	Feb	Mar	Apr
BMP test to Zeolite dosages evaluation						
Semi-continuous assays						
GHG emissions from the digestate.						

4.2.5. References

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5. GHG emissions reduction post-treatment strategies

5.1. S/T5 Nitrification-denitrification (NDN)

5.1.1. Introduction

Due to livestock feeding, animal manure is rich in nutrients, especially organic matter and nitrogen. These nutrients have traditionally been applied directly to the field as amendments or fertilizers. However, due to the high intensity of production of these streams, it is necessary to limit the organic input to soils, as excess nutrients end up being leached and affecting water bodies, especially aquifers. In addition, excess nutrients generate unwanted reactions that increase emissions of gases with a high greenhouse effect, such as N_2O and CH_4.

To reduce the organic matter and nutrient load of manure, different technologies can be applied, such as nitrification-denitrification (NDN). This technology is one of the most robust and most widely implemented. It has been widely implemented in urban and industrial wastewater treatment plants. This technology is based on the combination of bacteria with different biological processes, which transform organic matter and ammonium (the main nitrogen component of manure) into CO_2 and N_2, (compounds with less potential for pollution, both in the air and in water bodies).

The NDN process begins with an anoxic stage (in the absence of oxygen), where the organic matter in the influent comes into contact with the nitrates generated in the second stage, the aerobic phase, which are carried from the second stage to the first by a recirculation stream. The organic matter will serve as a carbon source for heterotrophic bacteria, and nitrate is the electron acceptor for the process. Because heterotrophic bacteria have a greater preference for oxygen, it is important to limit aeration and oxygen supply in the anoxic stage, otherwise the bacteria will use oxygen as an electron acceptor, and the nitrate will not be reduced to N_2.

In the second stage, the aerobic stage, the remaining organic matter is oxidized to CO_2. Ammonium is first oxidized to nitrite by ammonium-oxidizing bacteria (AOB) and then oxidized from nitrite to nitrate by nitrite-oxidizing bacteria (NOB).

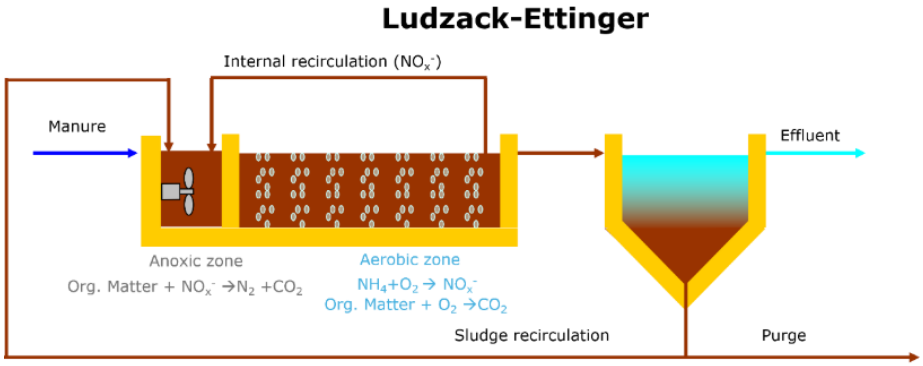

Figure 15. Diagram of the nitrification and denitrification process.

Although in theory organic matter is converted to CO_2 and ammonium to N_2, some complementary reactions may occur in the reactor that can lead to the emission of other gases. In the first zone of the reactor, the anoxic zone, due to the absence of oxygen, anaerobic reactions may take place, promoting the generation and subsequent emission of CH_4. On the other hand, due to aeration, and depending on the pH of the stream, the ammonium in the influent will be in the form of ammonia (NH_3), favoring its stripping. Finally, during the nitrification and denitrification process, reactions may occur that generate N_2O emissions.

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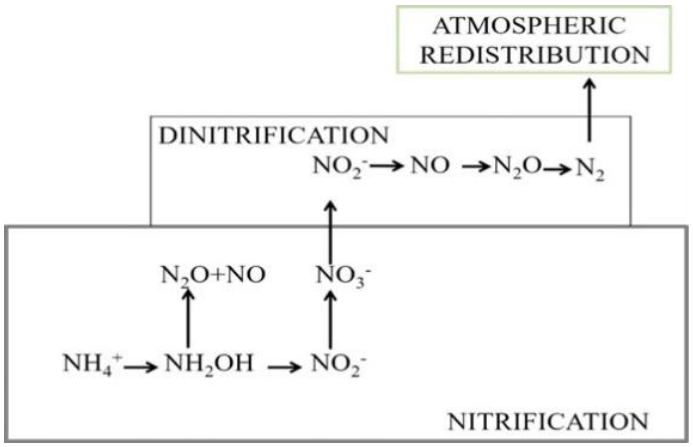

Figura 16. Intermediate reactions of the NDN process (Shafreen et al., 2021).

It should be noted that, in a conventional treatment plant, approximately more than 50% of GHG emissions are mainly due to diffuse N_2O emissions from the biological reactor (Rodriguez-Caballero et al., 2015). The rest of the GHG emissions are usually due to energy consumption (mainly from the supply of oxygen in the aerobic phase). Therefore, N_2O emissions should be the main parameter for monitoring diffuse sources in this type of biological reactor.

5.1.2. Methodology

The objective of the work plan is to determine greenhouse gas emissions under different operating parameters in order to identify which aspects have the greatest influence on these emissions.

First, it will be necessary to design a biological reactor in which to carry out the NDN process and which can include all aspects related to the measurement of GHG emissions. A working scale of 2-10 L has been proposed, taking into account the availability of real manure and the daily demand for manure from the reactor.

Once the reactor is available and the corresponding hydraulic tests have been carried out, a series of experimental tests will be carried out with synthetic water that simulates the quality of the manure that will be available. This experimental period will serve to adapt the biological sludge to the SBR reactor, while also allowing the GHG measurement methodology to be tested and improved.

After completing the tests with synthetic water, the reactor will be fed with real manure. It is expected to be fed with cattle manure from intensive farming. The operating parameters of this reactor will be modified in order to evaluate GHG emissions under different conditions. The emissions of N_2O, NH_3, and CO_2 will be monitored. The duration of each experimental period will be established based on the time it takes for the reactor to reach a stationary state. In addition, the performance of the reactor under these operating conditions will also be taken into account. Mainly, the nitrogen and organic matter content of the effluent will be measured. The associated energy consumption will also be considered in relation to the aeration of the aerobic phase.

Based on existing scientific knowledge, the following operating parameters will be modified: cell retention time, dissolved oxygen set point concentration, hydraulic residence time, and organic loading rate. For the latter parameter, the ratio of organic matter to nitrogen in the influent will be taken into account. In addition, given that the influent is expected to have a high organic load, the behavior of the reactor will also be evaluated working with the anoxic and aerobic phases separated in time, compared to the same process but working anoxically and aerobically simultaneously.

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5.1.2.1. *N2O measurement protocol*

As explained above, N2O emissions are usually approximated based on an emission coefficient that relates N2O emissions to the nitrogen load in the influent. However, although this value may be appropriate for an approximate value, it must be calculated in each scenario, considering that N2O emissions are one of the aspects that contribute most to the carbon footprint of WWTP. N2O emissions can be measured in two ways. On the one hand, a gas meter can be included, or a bag or dome can be placed on the water surface to accumulate the gases, so that their N2O concentration can be measured later. Knowing the concentration and velocity of the gases, the N2O emission can be determined. On the other hand, it is possible to measure the concentration of N2O in the liquid and then estimate its stripping rate, considering the aeration flow. In both cases, it is possible to obtain a concentrated measurement of N2O emissions into the atmosphere, but by measuring liquid N2O, more details about N2O production can be obtained. Due to the measurement in the aqueous phase, it will be possible to observe if there are variations in the anoxic phase, while if the gas were measured directly, only significant variations in those phases that are aerated would be measured, since the measurement depends on the strength of the stripping.

In order to determine N_2O emissions into the atmosphere, an adaptation of the model developed by (Bielefeldt & Stensel, 1999) to determine VOC emissions will be used. This adaptation of the model, taken from (Domingo-Félez et al., 2024), allows to determine N2O emissions in (gN/day) based on the following formula:

$$N_2O_{emission} = k_{LaN20} \cdot \left(N_2O_{liq} - N_2O_{liq,air}\right) \cdot V$$

Where V is the reactor volume, $N_2O_{liq,air}$ is the concentration of N_2O at the liquid-gas interface. Due to aeration of the medium, this value will be assumed to be close to zero. N_2O_{liq} is the concentration of N_2O in the liquid and k_{LaN20} is the mass transfer coefficient of N_2O. To obtain the value of k_{LaN20} Domingo-Félez et al. (2024) proposes the following correlation with the mass transfer coefficient value of O2:

$$k_{LaN20} = k_{LaDO} \cdot \sqrt{\left({}^{D_{N20}}/_{D_{DO}}\right)}$$

In this case, k_{LaDO} can be obtained based on different approximations depending on the type of aeration or the intensity of agitation. For the anoxic phase, without aeration, the k_{LaDO} data extracted from the study by (Díaz-Barrera et al., 2009) will be used, which relates this value to the agitation speed.

Agitation rate (rpm)	k_La (h^{-1})
260	22.3
300	39.0
340	47.2
400	56.5
450	63.2
500	65.5

During the aeration phase, data from Parakulsuksatid et al. (2000) will be used, which establishes a value of 340 h^{-1} for a stirred reactor with conventional aeration. This value is consistent with the literature review carried out by (Mariyana et al., 2018) of reactors with different aeration and stirring rates.

Reactor type	Gas Flow rate (mL/min)	Highest k_La (h^{-1}) Obtained for Oxygen
String film (SFR)	500	874.67
Stirred tank (900 rpm)	400	114
Stirred tank (1000 rpm)	5000	216
Bubble column	--	360
Airlift	--	360

It should be noted that this approximation of k_{LaDO} can be made due to the working scale. In an industrial environment, a profile of the gas velocity inside the reactor should first be made, as this will decrease as the measurement point moves away from the blowers. Therefore, in the case of a laboratory-scale reactor, an approximation of emissions can be made based on a single point, while in a larger-scale reactor it would be necessary to increase the number of measurement points in order to be able to include all the variability of the reactor.

To obtain the oxygen diffusion coefficient, the following bibliographic value has been taken: D_{DO} = 2.0 x 10^{-9} m^2/s (Xing et al., 2014) and for D_{N2O} = 1,77 x 10^{-9} m^2/s according to (Ying & Eimer, 2012). Both values are given for 25°C. If working at a different temperature, these values must be corrected.

With this information, the emission factor can be obtained for each operational parameter based on the nitrogen fed into the reactor over the course of a day or for a specific period of time (an entire SBR cycle or the aerobic or anoxic phase). This procedure also allows for a more accurate estimate of N2O emissions over a complete cycle.

$$EF = \frac{N_2O_{emission}}{TN_{Load}} \%$$

5.1.3. Results

It is known that the main GHG emissions in an NDN process are usually associated with N_2O emissions from biological processes (Daelman et al., 2013). Taking into account that this compound has a global warming potential 270-300 times greater than CO_2, it is important to note that during NDN, approximately 3% of N-N_2O is usually emitted for each N-NH_4 in the influent that is removed (Frutos et al., 2017) (Guo et al., 2018). However, this reference value is a general value that can be used in estimates, but it depends on different operating parameters.

One of the main factors affecting N_2O production is the concentration of dissolved oxygen. A low oxygen concentration promotes the formation of NH_2OH in both the nitrification and denitrification stages, increasing the amount of N_2O released. Moreover, as a result of the low content of organic matter during the aeration stage, heterotrophic denitrifying bacteria utilized the intercellular organic matter, PHA, in the denitrification process, leading to significant emission of N_2O (He et al., 2009). Furthermore, under low DO concentration, some autotrophic ammonia-oxidizing bacteria may utilize nitrate or nitrite as the electron acceptor in autotrophic denitrification processes, through which a considerable amount of N_2O could be generated (Kong et al., 2013). This means that a higher oxygen supply should reduce the presence of N_2O; however, greater aeration is associated with

higher energy consumption. Thus, a balance must be sought between performance, cost, and environmental impact.

On the other hand, the accumulation of NO_2 in the reactor can also promote the generation of N_2O (Kinh et al., 2017) (Figure 17). In some cases, either due to a low presence of organic matter or in order to reduce aeration costs, low hydraulic residence times are used, which can promote the accumulation of NO_2. Denitrification from NO_2 instead of NO_3 reduces both the organic matter requirements in the denitrification phase and the aeration requirements. However, when NO_2 accumulates, it has been observed that AOBs release N_2O to protect themselves from the presence of NO_2, when it is present in values that can be toxic or inhibitory to them (Zhou et al., 2011).

In relation to the presence of nutrients, it is also important to consider the COD/N ratio in the influent (Law et al., 2012). When the ratio between organic matter and nitrogen is insufficient (less than 4.7), N_2O generation can increase by up to 30% according to various authors, either because denitrification is not carried out completely or because several enzymes do not participate correctly, promoting reactions that trigger the formation of N_2O (Guo et al., 2018).

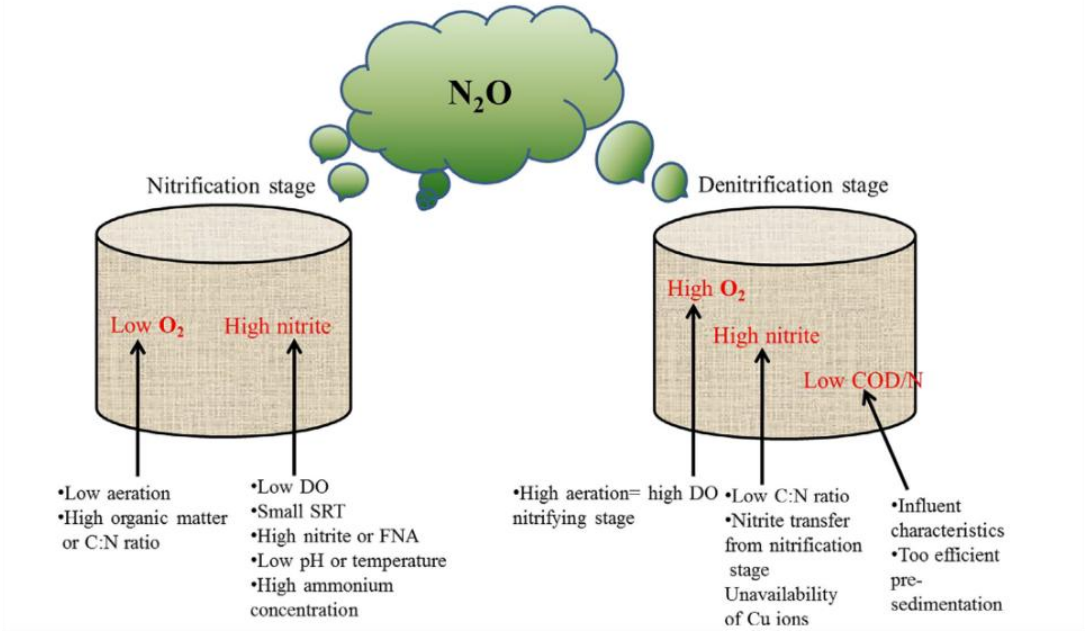

Figure 17. Different key parameters responsible for N2O emission (Thakur & Medhi, 2019).

Sludge residence time (SRT) is also involved in greater or lesser accumulation of N_2O. High SRT levels allow for a high active bacterial population, which reduces NO_2 accumulation levels (Campos et al., 2016). It is also possible that high SRT values can maintain K-strategist bacteria, which use resources more efficiently, reducing the intensity of complementary reactions that form N2O during the nitrification or denitrification process. For example, in the case of denitrifying bacteria, it should be noted that there are two large groups: the genus *Nitrospira*, which typically behaves like K-strategists, and the genus *Nitrobacter*, which behaves like R-strategists. The presence of each of these genera can be conditioned by the established SRT (Blackburne et al., 2007).

It is important to consider how all factors can interfere with each other, while having a significant impact on the potential effluent quality and energy consumption of a biological reactor. For example, when increasing the SRT of a reactor, blowers must work harder to ensure oxygen diffusion throughout the reactor. In addition, reactors with a high SRT often have areas with low oxygen

levels. For this reason, in addition to conventional processes, other authors have evaluated the NDN process simultaneously (SNDN). The advantages of SNDN are focused on its good purification capacity in a faster and more energy-efficient way. However, its operating conditions can cause high N_2O emissions (Kong et al., 2016). For this reason, the advantages and disadvantages of this type of reactor must be evaluated in greater detail, in comparison with conventional processes.

There are other aspects that can affect N_2O formation, but they are more difficult to control or manage, such as the availability of micronutrients such as copper. Copper is particularly important because the N_2O reductase enzyme produced by different bacteria requires copper as part of its structure. This enzyme is responsible for reducing N_2O to N_2. (Zhu et al., 2013) demonstrated that after supplementing the copper deficiency in a reactor, they were able to reduce N_2O emissions by 50-73%.

There are currently multiple lines of work underway to address the lack of information on N_2O emissions in reactors of different configurations. The need to find a reactor configuration that minimizes emissions and energy and reagent consumption while ensuring good effluent quality is becoming increasingly important.

5.1.4. Conclusions and next steps

Based on the information compiled, it is necessary to establish a protocol for measuring N2O emissions in order to estimate more accurately the greenhouse potential of an NDN purification process based on its operating parameters. The established protocol will make it possible to identify the moments of greatest N2O generation and differentiate them from the moments when N2O is emitted. The literature review reaffirms that the selection of operating parameters will make it possible to study the main factors that affect N2O production.

The following actions will be carried out over the coming months

- Start-up of the reactor and hydraulic tests
- Experimental tests with synthetic water and real sludge to adjust the emission measurement method
- Experimental tests with real manure
- Analysis of the results obtained

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5.2. S/T6: Partial nitritation/anammox (PN/AMX)

5.2.1. Introduction

The management of livestock slurry in intensive farming represents a significant environmental and economic challenge due to its high emissions of greenhouse gases (GHG) such as nitrous oxide (N_2O). Moreover, excessive untreated slurry application to land can lead to water pollution through nitrate leaching, soil degradation, ammonia (NH_3) volatilization, odour nuisance, and a considerable contribution to agricultural GHG emissions. These concerns are recognized at both the European Union and national levels, as reflected in policies such as the EU Nitrates Directive 91/676/EEC (Council Directive 91/676/EEC, 1991), which limits nitrogen application to protect water quality, and Spanish Royal Decree 988/2022 (Real Decreto 988/2022, 2022), which regulates monitoring and reporting of emissions from livestock farming. As environmental regulations become stricter, there is an increasing need for farms to adopt cost-effective and climate-friendly waste treatment solutions that ensure compliance while reducing their carbon footprint.

Traditional biological treatment technologies, such as nitrification-denitrification, effectively remove nitrogen but are energy-intensive due to their high aeration demand. In addition, they often produce significant amounts of N_2O, which has a global warming potential nearly 300 times that of CO_2 (GHG Protocol, 2024). This dual drawback (high operational costs and elevated GHG emissions) highlights the need for an alternative process.

Partial nitritation/Anammox (PN/AMX) is an innovative and energy-efficient biological process for removing nitrogen from wastewater and digestate. It involves two steps: first, aerobic partial nitritation (PN), in which approximately 50% of the ammonium is oxidized to nitrite by ammonia-oxidizing bacteria (AOB), and second, anaerobic ammonium oxidation (Anammox), where the remaining ammonium and nitrite are converted into nitrogen gas (N_2).

Compared to traditional biological nitrogen removal by nitrification-denitrification, the PN/AMX process achieves lower operational cost primarily due to its reduced oxygen demand, which decreases energy requirements. Furthermore, the absence of an organic carbon demand allows for the maximum conversion of organic matter into methane during anaerobic digestion, thereby enhancing energy recovery from biogas (Val Del Rio et al., 2019).

Despite these advantages, there is still limited knowledge about its performance with respect to greenhouse gas (GHG) emissions, particularly nitrous oxide (N_2O) (Zhao et al., 2024). Before full-scale application of PN/AMX to livestock slurry, several key aspects must be addressed: assessing the long-term stability and performance of the microbial populations under real operational conditions, reducing the relatively slow start-up and acclimation period of PN/AMX biomass (Pedrouso et al., 2023), and ensuring that adequately trained personnel are available for process operation and monitoring.

According to the Document of Agreement (DoA), this research aims to fill these knowledge gaps by studying PN/AMX in both one-stage and two-stage configurations, focusing not only on effluent quality but also on reducing GHG emissions. The objective is to optimize system performance under livestock slurry conditions, gain a better understanding of the long-term GHG dynamics, and provide a comprehensive environmental and economic comparison with conventional systems to support future on-farm implementation.

5.2.2. Methodology

5.2.2.1. Experimental setup

The experimental phase began with the operation of two lab-scale reactors in a two-stage PN/AMX configuration:

- Partial Nitritation (PN) reactor: an aerobic reactor inoculated with activated sludge from a Wastewater Treatment Plant (WWTP) in Santiago de Compostela. It was operated at hydraulic retention times (HRT) ranging from 1.0 and 0.7 days, treating a feed containing 1.0 g NH_4^+-N/L.
- Anammox reactor: an anoxic reactor inoculated with biomass from a previously anammox-enriched laboratory reactor. Operated at a fixed HRT of 1 day, with nitrogen concentrations from 0.6 to 1.0 g NH_4^+-N/L.

5.2.2.2. Abiotic tests for N2O production

Abiotic tests were conducted to assess the potential contribution of nitrite to generate N_2O emissions chemically under varying pH conditions and at room temperature. A nitrite solution (0.5 g NO_2^--N/L) was aerated at different pH levels (9.3 to 6.0), and the headspace gas was collected and analysed by gas chromatography to detect possible N_2O formation. No N_2O was detected in these assays, which suggests that any potential abiotic contributions to the emissions of this gas remained below the detection limit of the gas chromatograph used (Hewlett Packard 5890 Series II-TCD with Porapak Q column, detection range 0.1-100%).

5.2.2.3. GHG measurement protocol

For measuring GHG emissions from the bioreactor off-gas, an online gas analyzer (model URAS26 3020, ABB) based on non-dispersive infrared spectroscopy was installed. This technology detects gases by measuring the absorption of infrared light at specific wavelengths characteristic of each

compound. The analyzer allowed continuous monitoring of the reactor headspace and provided real-time data on the concentrations of key GHGs such as CO_2, CH_4, and N_2O. The system has a detection limit < 0.4% of the measurement range under normal operating conditions, allowing for precise quantification of emissions during reactor operation.

5.2.3. Results

The first experimental phase focused on enriching biomass capable of treating the high nitrogen loads typically found in digestate from livestock slurry.

In the PN system, a synthetic feed containing 1 g NH_4^+-N/L was used for GHG quantification as baseline and methodology validation. To progressively adapt the biomass to this high ammonium concentration, the reactor was operated as a sequencing batch reactor for 74 days under three HRTs. In Stage I, the reactor was operated at an HRT of 0.5 d, followed by Stage II and III at 1.0 and 0.7 d, respectively. This strategy successfully promoted the selection of AOB. By the end of Stage III, the reactor reached a stable performance, achieving an Ammonium Oxidation Rate (AOR) of 57.4 ± 3 %, indicating effective PN under the tested conditions (Figure 18). This value is close to the theoretical 55 % conversion of ammonium to nitrite required for anammox coupling, ensuring that sufficient ammonium availability while minimizing nitrate formation.

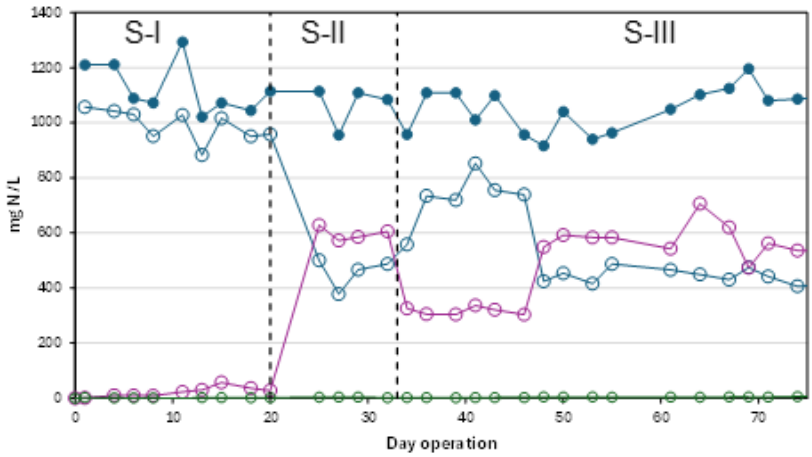

Figure 18. Evolution of nitrogen compounds: concentration of NH4+ in the influent (solid blue), and NH4+ (empty blue), NO2- (empty violet) and NO3- (empty green) in the effluent in mg N/L.

For the Anammox reactor, a different enrichment strategy was applied. The HRT was maintained at 1 day, while the influent nitrogen concentration was gradually increased until a Nitrogen Loading Rate (NLR) of 1 g N/(L·d) was reached. As shown in Figure 19, from approximately day 125 of operation, the reactor achieved a stable performance, with a Nitrogen Removal Efficiency (NRE) of 86.5 ± 4 % at the target NLR.

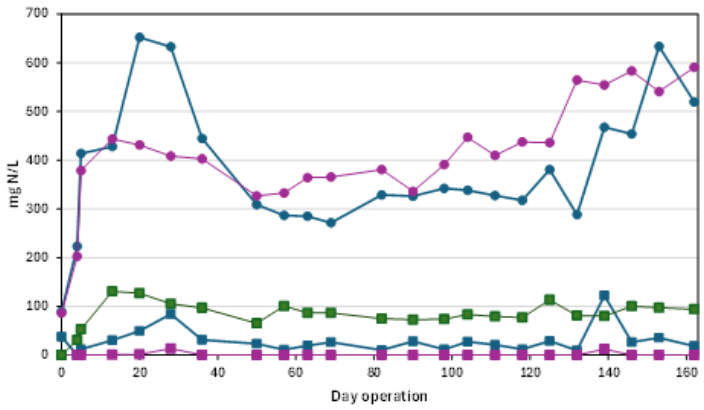

Figure 19. Evolution of nitrogen compounds: concentration of NH4+ (blue circle) and NO2- (violet circle) in the influent, and NH4+ (blue square), NO2- (violet square) and NO3- (green square) in the effluent in mg N/L.

These preliminary results demonstrate the successful start-up and enrichment of both PN and Anammox reactors. The enriched biomass showed the capacity to treat high-strength nitrogen streams, confirming that the systems are suitable for further testing with real anaerobically digested slurry and subsequent evaluation of GHG emissions.

In addition, the abiotic tests showed that no detectable N_2O was produced from nitrite in the solutions in any of the tested conditions. This suggests that chemical nitrite oxidation is not a significant contributor to GHG in the system. If such abiotic emissions were relevant, they could hinder the feasibility of the two-stage PN/AMX systems, where nitrite is accumulated in the liquid phase. These results reinforce the suitability of both PN/AMX configurations and highlight the need to focus on the biological in the following experimental phase.

5.2.4. Conclusions and next steps

In the first phase of this project, the successful development of two-stage PN/AMX system enriched with biomass adapted to high nitrogen loads was achieved. These systems demonstrated stable performance under synthetic influent and are now being used to generate the GHG emissions baseline before transition from synthetic feeding to real anaerobically digested slurry as substrate. The abiotic assays confirmed that chemical nitrite oxidation does not contribute significantly to N_2O emissions under the tested conditions. This result is particularly relevant for two-stage PN/AMX configurations, where nitrite accumulation occurs, as it ensures that both one-stage and two-stage systems remain environmentally feasible.

Moreover, the following steps will include:

- Monitoring GHG emissions (CO_2, N_2O, CH_4) using online methods that are already available and develop a protocol to quantify the emissions of NH_3.
- Applying digestate from livestock slurry.
- Comparing the environmental performance of single-stage versus two-stage PN/AMX configurations, considering both nitrogen removal from the liquid phase and GHG.

These results will contribute to the development of a low-emission, cost-effective strategy for manure management, contributing to both regulatory compliance and climate change mitigation. No deviations from the DoA are anticipated.

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6. GHG emissions reduction strategies at the level of application

6.1. S/T7: Manure fertigation

6.1.1. Introduction

Ammonia (NH_3) volatilisation after slurry application represents one of the main indirect sources of greenhouse gas (GHG) emissions in agriculture, since nitrogen lost as NH_3 contributes to the formation of nitrous oxide (N_2O), a gas with high global warming potential. Reducing NH_3 emissions therefore improves both nutrient use efficiency and the climate footprint of livestock systems. Within NUTRITIVE, ARESA leads the assessment of NH_3 emissions in maize and grassland systems under contrasting slurry application techniques: conventional broadcast spreading and injection. The objective is to quantify the differences in volatilisation patterns and to evaluate potential trade-offs with crop productivity.

6.1.2. Methodology

The quantification of NH_3 volatilisation after slurry application will be carried out using ALPHA® passive samplers (Adapted Low-cost Passive High Absorption), following the principles of the VERA Test Protocol for Measurement of Gaseous Emissions from Land Applied Manure (2009). ARESA has prepared a full measurement protocol adapted to farm conditions in Galicia, which has been reviewed by ILVO. The summary below outlines the main elements of the methodology; the full protocol is available for consultation by project partners or stakeholders who require detailed technical specifications.

6.1.2.1. Experimental design

- Trials are performed in commercial fields (approx. 0.4 ha experimental plots within larger parcels).
- Each plot is divided into three subplots corresponding to the treatments:

1. Conventional broadcast application,
2. Injection of slurry,
3. Broadcast application with additive.

- A central mast with 5 ALPHA® samplers is placed in the middle of the treated subplot (heights: 0.25–3.3 m).
- A background mast with 3 samplers is positioned upwind (~50 m), ensuring no other NH_3 sources within 300 m and no obstacles closer than 10× their height.
- Meteorological data are continuously collected with a portable weather station.

6.1.2.2. Sampling procedure

- ALPHA® samplers are impregnated with acid solution, dried, and assembled prior to deployment.
- Samplers are installed with protective shelters to avoid rain and direct radiation.
- Exposure lasts 96 h per campaign: two replacements in the first 24 h, then one per day (total of 5 sampling windows).
- After exposure, samplers are sealed and transported at <4 °C until analysis.

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6.1.2.3. *Laboratory analysis*

- NH_3 captured on filters is extracted and quantified as NH_4^+ by ion chromatography or continuous autoanalyzer.
- Results are expressed as concentrations (µg m^{-3}) per exposure period.

6.1.2.4. *Emission calculations*

- Emission fluxes are calculated by ILVO using the Integrated Horizontal Flux (IHF) method, integrating concentration profiles, wind speed, and fetch distance over successive intervals to obtain cumulative emissions (kg N–NH_3 ha^{-1} at 96 h).

6.1.2.5. *Additional variables*

- Soil: granulometry classification and nutrient content before and after application.
- Meteorology: wind speed/direction, air temperature, humidity, precipitation. Experiments are only scheduled during periods with stable wind conditions and no rain within six hours of application.

This methodology ensures traceability, comparability, and scientific robustness, in line with VERA requirements while adapted to practical farm conditions in Galicia.

6.1.3. Results

Literature consistently shows that injection of slurry can reduce NH_3 emissions by 30–90 % compared with surface broadcasting, depending on soil moisture, crop type, and weather (Artetxe et al., 2013; Misselbrook et al., 2014; Sánchez-Martín et al., 2020). Injection is thus recognised as one of the most effective mitigation practices to limit atmospheric nitrogen losses.

However, several studies have highlighted potential trade-offs: while NH_3 volatilisation decreases strongly, the risk of increased N_2O emissions may arise because injection creates zones with high nitrogen and carbon availability under reduced soil aeration, favouring denitrification (Sommer et al., 2009; Meijide et al., 2020; Hou et al., 2022). Reported outcomes vary widely with soil texture and moisture, suggesting that the net climate effect of injection depends on local conditions.

Importantly, there is a lack of field evidence under Atlantic conditions, such as those of Galicia, where high rainfall and relatively cool temperatures could alter both NH_3 volatilisation and subsequent N_2O formation. This gap justifies the need for our measurement campaigns to provide region-specific data to underpin recommendations for sustainable slurry management.

6.1.4. Conclusions and next steps

The methodology has been consolidated, and the next steps involve:

- Acquisition of all necessary equipment (ALPHA samplers, meteorological station, consumables).
- Contact with landowners of selected plots to secure agreements for experimental campaigns.
- Coordination with contractors and farmers with slurry injection equipment.
- Launch of first measurement campaigns (early 2026) aligned with manure application schedules.

This work will deliver quantitative evidence of emission reductions achievable through injection in Galicia, a region where such practices are rarely implemented at commercial scale.

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6.1.5. References

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6.2. S/T8: Additives application

6.2.1. Introduction

Besides application technique, the use of slurry additives represents another strategy to mitigate gaseous emissions. Additives can reduce NH_3 volatilisation, limit H_2S emissions, improve slurry homogeneity, and preserve the agronomic value of manure. Within NUTRITIVE, ARESA explores the potential of additive use under farm conditions, focusing on innovative commercial formulations.

6.2.2. Methodology

We are evaluating Farmodor C, a multicomponent biological product marketed by Nando. The product contains *Bacillus* strains, ammonia- and sulfide-oxidising bacteria, fungi, enzymes, and plant extracts. It is applied either in barns, storage tanks, or directly to slurry reservoirs, with recommended doses ranging from 15 g/m³ in cattle slurry tanks to 30 g/m³ in pig farms (applied every 10 days). The plan is to test Farmodor C under controlled farm-scale conditions, first in storage tanks and later in field applications, quantifying its effect on NH_3 emissions using the same ALPHA–IHF methodology.

The measurement protocol for additives follows the same principles as that used for application techniques. ALPHA® samplers are deployed around the treated plots to quantify reductions in NH_3 concentrations, while meteorological parameters are continuously recorded to contextualize the measurements. Laboratory analysis of the collected samples is then combined with the

meteorological data to generate validated concentration datasets. Using these inputs, ILVO performs the emission calculations, enabling a robust comparison between treated and untreated slurries.

6.2.3. Results

Farmodor C has demonstrated the following benefits in commercial trials and case studies:

- Reduction of NH_3 and H_2S emissions by up to 80 % (typical range 25–80 %).
- Decrease in unpleasant odours, improving on-farm working environment.
- Reduced crust formation in slurry storage, facilitating mixing and reducing agitation needs.
- Preservation of nitrogen in slurry (up to 25 % less N loss), enhancing fertiliser value for crops.

6.2.4. Conclusions and next steps

Preliminary evidence suggests Farmodor C can significantly reduce gaseous losses while improving slurry handling and agronomic quality. The next steps for ARESA include:

- Continuing discussions with Nando representatives to define precise dosing strategies for Galicia.
- Conduct on-farm testing of the additive in at least one cattle slurry storage tank to validate its performance under local conditions and subsequently integrate additive treatments into field emission trials in parallel with application technique comparisons.

This dual approach (application method + additive use) positions ARESA at the forefront of emission mitigation strategies in manure management, generating robust data for farmers, policymakers, and the scientific community.

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