Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 30 June 2026
https://doi.org/10.14295/CS.v17.4393

Integrated optimization of poultry waste conversion into biofertilizer and biogas for sustainable tomato cultivation

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Abstract

Sustainable tomato production increasingly requires efficient and environmentally responsible fertilization strategies. In this context, poultry waste valorisation through anaerobic digestion (AD) offers a promising route to produce nutrient-rich biofertilizers while generating renewable energy. The present study investigated the combined effects of key AD factors on both biogas yield and digestate quality intended for horticultural use. Four factors were examined using a 2^4 factorial design: temperature, moisture, molasses addition, and *Aspergillus niger* inoculation, resulting in sixteen combinations. The mixtures underwent a 21-day methanization process. Comprehensive physicochemical and nutritional analyses were conducted, followed by a tomato fertilization test to assess agronomic performance. Fungal inoculation exerted the strongest positive effect on process efficiency. Temperature enhanced potassium availability, whereas excessive moisture limited total organic carbon degradation. Mixture M12 produced the highest biogas yield (>17,000 mL) and exhibited balanced nutrient contents (N 1.45%, P 1.98%, K 2.5%). Its digestate improved tomato growth, outperforming the control and approaching the performance of chemical fertilizer. Optimisation of the AD process therefore supports sustainable horticultural production while contributing to waste reduction and renewable energy recovery.

Keywords: anaerobic digestion, biofertilizer, circular agriculture, tomato production

Introduction

Poultry meat has long been considered one of the most widely consumed sources of animal-based protein globally. The very high demand for this white meat has led to a significant increase in farming and industrial production worldwide, with an estimated 151.4 million tons in 2025, while experts predict the number will rise to 173 million tons by 2034 (OECD/FAO, 2025). This sharp increase is mainly due to several factors, including global population growth, rising incomes, urbanization, and people's belief that poultry meat is a healthier source of protein than red meat and is also less expensive (FAO, 2023). This global intensive production rate has generated a huge amount of organic food waste, including litter, feathers, viscera, blood, and bones, as well as other processing residues. Therefore, the poultry production systems have been defined as one of the major contributors to nutrient pollution that affects aquatic ecosystems, with many studies proving that high levels of nitrogen and phosphorus can be found in surrounding watersheds caused by the release of these nutrients from poultry waste through runoff and leaching, thus leading to many environmental issues such as eutrophication and oxygen depletion (Nahm, 2007; Gržinić et al., 2023). Poultry waste, therefore, triggers an urgent alert, showing that if not treated effectively, it can represent a significant global source of pollution, affecting air, soil, and aquatic systems.

The Moroccan Interprofessional Federation of Poultry reported that Morocco produces approximately 635,000 tons of poultry meat and 5.5 billion eggs per year, making this sector one of the most dynamic food industries nationally (FISA, 2020). However, the poultry industry generates significant amounts of waste, including wastewater and solid waste which consists of litter, excrement (manure), animal feed, feathers, hatchery

Received: 02 March 2026
Accepted: 06 March 2026

waste (dead embryos, sterile eggs, dead fetuses, and late ovulations), shells, sludge, and slaughterhouse waste (offal, blood, and feathers) (Ajmani et al., 2025). In this context, it is important to note that the Moroccan poultry sector has made great strides, mainly thanks to investments made under the Green Morocco Plan. These initiatives have helped to modernize practices and mitigate environmental impacts, demonstrating the effectiveness of strategies focused on sustainable development (Mathez & Loftus, 2023).

Poultry waste can be converted into several by-products for use in a variety of industries using the circular approach. One of the best circular techniques for managing organic waste is anaerobic digestion (AD). This approach produces methane from a variety of organic wastes, including manure, organic food, sewage sludge, and agricultural slurry, offering a flexible and sustainable energy source (Kitessa et al., 2022). Because microbial metabolisms are involved, anaerobic digestion provides efficient waste treatment and produces useful methane. Biogas applications have grown beyond the generation of heat and power to include car fuel and natural gas-like functions. Landfills, composting, incineration, and agricultural use are examples of traditional disposal techniques that face restrictions due to environmental and health issues (Abdulkarim et al., 2024). By maximizing the production and use of biogas, anaerobic digestion emerges as a cost-effective and environmentally friendly alternative that can drastically lower operating costs for wastewater treatment facilities or even produce income through biogas sales. Additionally, this process can also generate a digestate, a byproduct of AD, which is rich in nutrients that can be used as a biofertilizer.

AD improves soil fertility by producing useful residual digestate biofertilizers. In an effort to address issues with chemical fertilizers and accelerate the shift to sustainable energy practices, it highlights the need to address institutional, financial, technological, and societal impediments. Biofertilizers are inexpensive, sustainable, and environmentally friendly since they use the naturally occurring biological system to mobilize nutrients. AD not only develops a new source of green energy in the form of biogas but also produces stable digestates with high performance, proving the case for the circular economy (Foughal et al., 2024; Doublali et al., 2025).

Despite the growing number of studies on AD and poultry waste valorization, there is no single study to date that has simultaneously assessed the combined effects of *A. niger* inoculation, temperature, moisture, and molasses addition on poultry waste digestion and studied the synergic effect between these factors and their influence on the biogas yield from one side and the biofertilization potential from the other side. This present work investigates the effect of these key factors on the performance of poultry waste AD. Using a 2□ factorial design, sixteen mixtures combining these variables were tested over 21 days to evaluate biogas production and substrate degradation (pH, dry matter, and conductivity). Physicochemical and microbiological analyses were performed to identify the optimal conditions enhancing methane yield and digestate quality. Additionally, to evaluate the AD efficiency and nutrient recovery potential, several comprehensive analyses of total organic carbon (TOC) and nutrient contents (N, P, K) were made. Eventually, the best-performing digestate, characterized by balanced nutrients, a suitable C/N ratio, and compliance with the standard NF U 44-051, was further validated through germination and fertilization analyses applied to tomato crops, confirming its potential as an efficient and safe biofertilizer.

This research not only highlights the potential of using a multifactorial approach in poultry waste digestion but also reinforces the notion that fungal inoculation, particularly with *A. niger*, may offer a biotechnological lever to enhance AD performance, especially when combined with other factors such as Molasses, adequate temperature, or a suitable level of moisture. The observed trends offer a foundation for optimization in full-scale systems and justify further exploration into fungal-assisted valorization of Moroccan poultry waste.

Materials and Methods

Substrate Preparation and Characterization

Poultry slaughterhouse residues are broadly available in the Agro-industrial sector in Morocco. And as they are rich in organic content, they are considered suitable for a biotransformation process, so these residues were then chosen as the main substrate for the AD process in our study. **Figure 1** shows a schematic representation of the initial substrate preparation and assessment.

Blood, feathers, heads, feet, and bone fragments were sourced directly from nearby industrial slaughterhouses. These elements together represent an important amount of poultry processing by-products. 2 kg of this waste was collected and stored in the laboratory's refrigerated environment. A laboratory grinder was used to grind the samples until they formed a homogeneous and consistent paste to ensure homogeneity and facilitate further analysis. Part of the prepared material was set aside for initial physicochemical and biochemical characterization (e.g., pH, dry matter, volatile solids, and

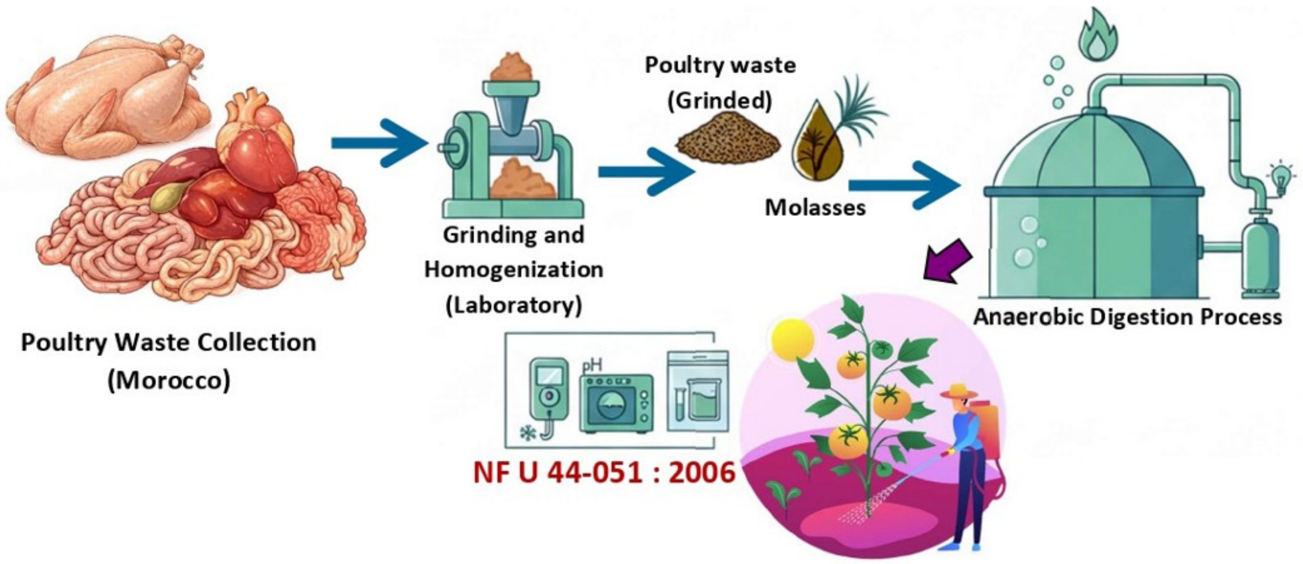

Figure 1. Representation of the Initial Substrate Preparation and Characterization Approach

nutrient content), while the other part was intended to be incorporated directly into the formulation of experimental mixtures for methanization. The samples were vacuum-sealed and stored at freezing temperatures until needed.

Sugar cane molasses was selected as the AD co-substrate in order to enhance the mixtures' nutritional balance, specifically the carbon/nitrogen (C/N) ratio. Molasses, a common by-product of the sugar industry, is a great supplement for AD because it is high in minerals and readily fermentable carbohydrates. It is essential to include poultry waste because it is typically high in nitrogen and has a low C/N ratio, which could prevent ammonia from being produced if digested on its own. Therefore, the goal of mixing molasses and poultry waste was to produce the optimal substrate composition to maximize biogas production, guarantee process stability, and promote microbial activity. The reference strains of *A. niger* (11G323A) were employed in this study.

Table 1 presents different levels of physicochemical and nutritional analyses of the initial substrate-poultry residues.

This waste, with its high protein (16.6%) and nitrogen (1.05%) content, moderate moisture content (51.91%), and nearly neutral pH (7.1), presents the best conditions for microbial growth. Molasses, on the other hand, is considered a rich and easily digestible carbon source since it contains high levels of moisture (73%) and total sugars (46.7%) and has a slightly acidic pH (6.7). In fact, molasses would balance the medium in terms of mineral availability since it contains less phosphorus (0.10%) and more potassium (1.05%) than poultry organic residues, which show low values for both phosphorus (0.196%) and potassium (0.13%). Besides, molasses has a higher C/N ratio (46.7) than poultry (21.9), and the combination of both becomes more balanced, favorable for microbial activity and AD efficiency. Electrical conductivity values indicated that the ionic content is higher in molasses (4.81 mS/cm) compared to poultry (2.2 mS/cm), which could affect osmotic conditions during the AD process. These features together give more reason for their use as a balanced microbial fermentation medium.

Preparation of fermentation mixtures using factorial design 2(4-0) with four factors

A factorial experimental design was used for the investigation of interaction effects in this study, and their effect on fermentation results. A factorial design of 2^4 was chosen, with “2” indicating that each factor is tested at two different levels, and "4" indicates the number of factors used in the study. This design allows for the evaluation of the effect of each factor and the effect of the interaction between them, thus providing a better understanding of their influence on the AD process. Four key factors included in this experiment were the following: (1) inoculation with *A. niger*; (2) fermentation, temperature, either mesophilic (37°C) or thermophilic (45°C), (3) fermentation mode, either liquid or solid; and (4) the addition of sugarcane molasses, either present or absent. Finally, 16 mixtures were obtained by combining these four factors with their respective levels, and each combination represents a unique experimental condition (**Table 2**).

This factorial approach offers several advantages: maximization of the data obtained from a limited number of experiments, the study and identification of synergistic or antagonistic interactions between different factors, and the determination of optimal conditions for an

Table 1. The Initial physico-chemical and nutritional characterizations of the organic by-products used.

Parameter	Poultry	Molasses
pH	7.11 ± 0.06	6.7± 0.03
Conductivity (mS/cm)	2.2 ± 0.11	4.81 ± 0.15
MS %	51.91 ± 0.41	73.00 ± 0.36
TOC %	22.98 ± 0.34	37.00 ± 0.26
TN %	1.05 ± 0.05	0.80 ± 0.02
P %	1.6 ± 0.07	0.10 ± 0.00
K %	0.13 ± 0.02	1.05 ± 0.03
C/N Ratio	21.9	46.7
Proteins %	16.6 ± 0.12	0.00 ± 0.00
Total Sugar %	2.5 ± 0.09	46.7 ± 0.45

Table 2. Experimental design represented 2(4-0) for 16 mixtures

Mixture	Inoculated by *A. niger* (AN or without)	T°C (Thermo or Meso)	H% (SSF or LSF)	Molasses (with or without)
1	Without	Meso	LSF	Without
2	AN	Meso	LSF	Without
3	Without	Thermo	LSF	Without
4	AN	Thermo	LSF	Without
5	Without	Meso	SSF	Without
6	AN	Meso	SSF	Without
7	Without	Thermo	SSF	Without
8	AN	Thermo	SSF	Without
9	Without	Meso	LSF	With
10	AN	Meso	LSF	With
11	Without	Thermo	LSF	With
12	AN	Thermo	LSF	With
13	Without	Meso	SSF	With
14	AN	Meso	SSF	With
15	Without	Thermo	SSF	With
16	AN	Thermo	SSF	With

effective AD. This type of experimental design provides a systematic and reproducible method of effectively investigating the interaction of the factors. studied - in our case, the inoculum, temperature, substrate composition, and fermentation mode – and their influence on the efficiency of the bioprocess. A quantity of 200 g of ground waste was used for each experimental digestate, which was made in a supervised environment following the factorial design approach. Then, 1% of the mixture was inoculated with *A. niger*.

Mixtures meant for liquid-state fermentation (LSF) were regulated to maintain less than 15% dry matter and roughly 85% humidity to guarantee ideal conditions for wet AD (Raposo et al., 2012). According to the experimental plan, fermentation was carried out in either mesophilic (37°C) or thermophilic (45°C) conditions. Additionally, to improve methanization and evaluate its impact on the production of biomethane, 12% sugarcane molasses was added to specific mixtures. For the factorial evaluation, each prepared mixture was then put under the specified experimental conditions. A 500 ml Erlenmeyer flask was filled with 200g of waste. The digester had a thermostatic bath that allowed the temperature to be set at 37°C or 45°C in the bioreactor (mesophilic or thermophilic fermentation) and a hole where a capillary was fixed, which carried the biogas from the tank to the test tube. The displaced liquid method was used to measure the amount of biogas produced (**Figure 2**).

The prepared mixtures underwent a meticulously regulated 21-day methanization process. Each mixture's temperature was routinely checked, and the organic matter was periodically homogenized to maintain an even distribution and maximize the methanogenic bacteria's activity. Until biogas generation stabilized, daily measurements of biogas production and mixture weight changes were documented.

Physicochemical and nutritional analysis

A pH meter (Fisher Scientific, Basic AB15) was used to measure each test's pH at the start and finish of the AD process in accordance with the international standard ISO 10390:2005. A conductivity meter (HANNA instruments, EC215) was used to measure the conductivity following ISO 11265:2016. The dry matter (DM) was determined by steaming at 60°C for 24 hours (Lovergrove, 1966). The total organic carbon and nitrogen were measured using a catalytic thermal oxidation at high temperature TOC analyzer (Model: TOC-L Shimadzu, Japan) (Taiek et al., 2014). The phosphorus was measured using the phosphomolybdate analytical method through a UV spectrophotometer (Mihajlovic *et al.*, 2007). The potassium, as well as the content of the metallic elements (cadmium, nickel, copper, and lead), were all measured using ICP-OES plasma emission spectrometry (iCAP PRO series, Thermo Fisher Scientific) at the Pasteur Institute of Morocco.

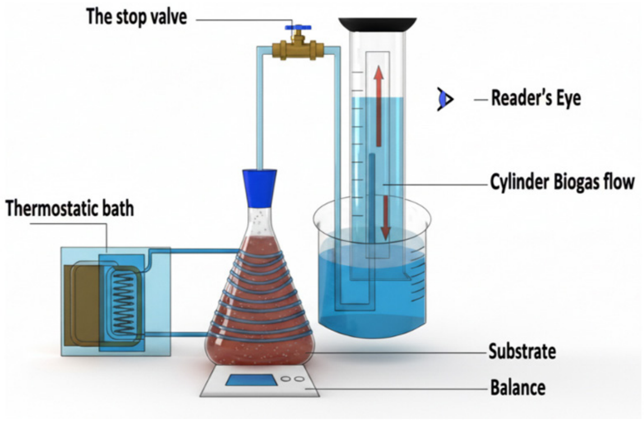

Figure 2. Schematic presentation of the reactor of the anaerobic digester.

Microbiological Analysis

Prior to and following the waste's anaerobic biotransformation, microbiological analysis was carried out in accordance with NF U 44-051 guidelines. A MacConkey agar was used to measure the presence of *Escherichia coli*, a hygiene indicator. While Salmonella was detected using the SS medium. Two Petri dishes of these already autoclaved media were used for the sterile inoculation of each mixture, which was subsequently incubated for 24 to 48 hours at 37°C. The colony-forming units (CFU/g) of the distinctive colonies that were seen were counted in order to record the results (Doublali et al., 2025).

Statistical Analysis

Principal Component Analysis (PCA) was used for statistical analysis. This statistical destinated to determine the most statistically significant factor with the highest impact on the different mixtures, through the estimation of their influence and interaction. The STATISTICA software was used to create the graphical representations.

Germination Test

5ml of the biofertilizer filtrate was put onto double-lined Whatman No. 1 filter papers in sterile Petri dishes in order to evaluate its toxicity. For statistical reliability, ten tomato (Solanum lycopersicum L.) seeds that had been surface-sterilized were distributed equally among three replicates per treatment in each dish. Instead of using the filtrate, sterile distilled water was used to create a control set. For seven days, the dishes were sealed and incubated at 25 ± 2 °C with a 12-hour light/12-hour dark cycle. The germination percentage and seedling vigor were then assessed using the following formula (Castañares & Bouzo, 2019):

$$G\% = \frac{\text{Number of germinated seeds}}{\text{Number of tested seed}} \times 100$$

Fertilization Test

In the fertilization test, tomato seeds (Solanum lycopersicum L.) were planted in pots 6 cm deep, 2-3 cm deep, and then covered with soil. Centrifugal phase separation was used to separate the chosen digestate into liquid and solid fractions so that their potential for fertilization could be compared. Measured trays with four columns and five wells (36 cm²) each were used in the experiment. Column 2 was given soil supplemented with the commercial fertilizer, while Column 1 served as the control and contained only soil. 0.054 g of the solid fraction and an equivalent dosage of the liquid fraction were added to columns 3 and 4, respectively, as shown in **Figure 3**. Every day, all of the containers received irrigation, guaranteeing steady moisture levels for seed germination and early growth evaluation (Hadidi et al., 2020).

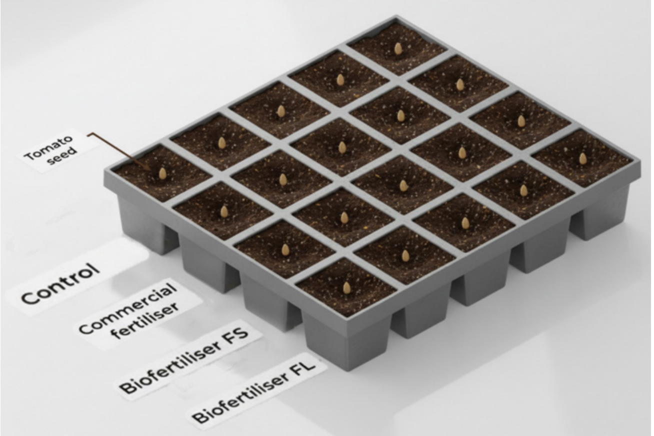

Figure 3. Simplified Three-Dimensional Illustration of the fertilization test; FS: solid fraction; FL: liquid fractio

Pigment extraction and spectrophotometric quantification of chlorophylls and carotenoids

Fresh leaf tissue is ground in 90% acetone, and the extract is clarified by centrifugation or filtration. The clear supernatant is then read in a UV spectrophotometer at 663, 645, and 470 nm using 90% acetone as a blank. The absorbance values are applied to standard equations (Lichtenthaler & Wellburn, 1983/1985) to calculate chlorophyll a, chlorophyll b, and carotenoids, and the results are expressed per gram of fresh weight (Lichtenthaler et al.,1985).

$$\text{Pigment}\left(\mu\frac{g}{gFW}\right) - \frac{C \ x \ V}{W}$$

Where:

C = concentration from equations (µg/mL)
V = final extract volume (mL)
W = sample fresh weight (g)

Results and Discussion

Evolution of Biogas and Digestate Mass

The cumulative biogas output variations (in milliliters) for sixteen distinct substrate combinations (M1–M16) throughout a 21-day AD period are shown in **Figure 4**a. The curves show three different phases that are frequently observed in the kinetics of AD. All mixtures exhibit a lag phase in the first few days (Days 1–5), characterized by little gas evolution as microbial consortia adjust to the substrates and start enzymatic hydrolysis. After that, there is an exponential production phase (Days 6–13) that is marked by sharp increases in gas volume. This corresponds to active acetogenesis,

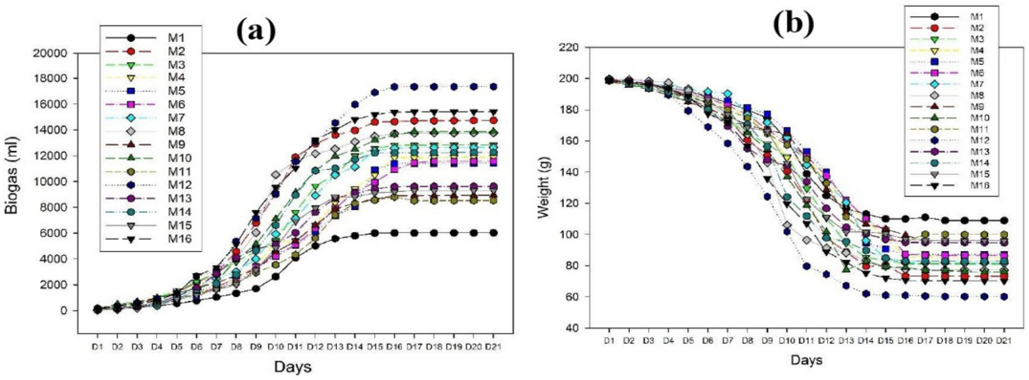

Figure 4. (a) Kinetics of cumulative biogas production; (b) kinetics of mass evolution for the 16 mixtures.

acidogenesis, and methanogenesis, which are processes that convert easily biodegradable organics into CO_2 and CH_4. The last stage, which starts on Day 14, shows a plateau in biogas production, which could be a sign of process stabilization or substrate exhaustion.

The presented results also showed that M12 has the highest biogas yield (>17,000 mL), which makes it the best-performing mixture. This digestate is formed from a combination of the studied factors (inoculated with *A. niger*, enriched with molasses, processed under thermophilic conditions, and solid-state fermentation (SSF). These factors are working in synergy to improve hydrolysis, substrate degradation, and methane production. M16 has presented a similarly high biogas yield, knowing that the only difference between this mixture and M2 is the moisture level, as M16 AD was made under a LSF. This means that the combination of the fungal inoculum with molasses and high temperature can still give important results, whether the medium is in a solid or liquid state. On the other hand, M2 benefits from the use of *A. niger* under mesophilic conditions in a LSF and without molasses, but it still achieves a good biogas yield, demonstrating that *A. niger* on itself remains able to strongly influence the biogas production, confirming the idea that fungal inoculation is a considerable factor in boosting biogas production (Hadidi et al., 2021). M8 and M10, which are both inoculated under mesophilic conditions, have performed moderately high, and this can probably be attributed to the absence of molasses, which has slightly limited total biogas generation. M8 is in SSF, while M10 is in LSF, which reflects that the AD process may work effectively in both cases if it is supported by the fungal inoculation. M3, M7, M14, M4, M6, and M5 produced around 11,000 mL to 12,800 mL, which makes them moderate-yield mixtures. M14 and M6 are inoculated with *A. niger* and molasses, but had SSF setups, which means that possibly this formulation with this setup led to carbon overload or substrate limitation, which causes a lower methane potential.

On the other hand, M3 and M7 did not have an inoculum and instead functioned in a thermophilic environment, which may have sped up the initial breakdown but resulted in inhibition later on because of heat stress or the buildup of volatile fatty acids (Meegoda et al.,2018). Likewise, M5 and M4, which are either uninoculated or thermophilic, demonstrate how the lack of fungal preconditioning can limit gas production at all temperature levels. M13, M15, M9, and M11 performed below average in the lower end of the spectrum. Complete digestion was probably limited by the lack of *A. niger* or mismatched SSF/LSF conditions, even though some of these were molasses-supplemented. The least efficient formulation is M1, which has a cumulative yield of less than 6,000 mL. The lack of all stimulatory stimuli in this mesophilic LSF system, which was devoid of the fungal inoculum and molasses, probably led to a poorly balanced C/N ratio, sluggish hydrolysis, and little microbial activity because of substrate recalcitrance or microbial inhibition.

As shown in the biogas results, the 5 mixtures with the highest biogas yield were all inoculated with *A. niger*, while the 5 mixtures with the lowest biogas yield had no inoculation, which reflects the clear positive effect of the fungi on the biogas yield. This species is known for the release of several enzymes that enhance substrate degradation and pre-hydrolysis, and its presence is consistently linked to higher methane production. These findings are also confirmed by the study of Ferde et al (Ferde et al., 2020).

3 of 4 best mixtures contained molasses, which indicates that he may be working in synergy with *A. niger*; however, molasses was also present in several low-performing mixtures without fungal inoculation, suggesting that molasses by itself may not be enough to promote biogas yield as strongly as if *A. niger* is present in the mixture. For instance, M1 which contains molasses

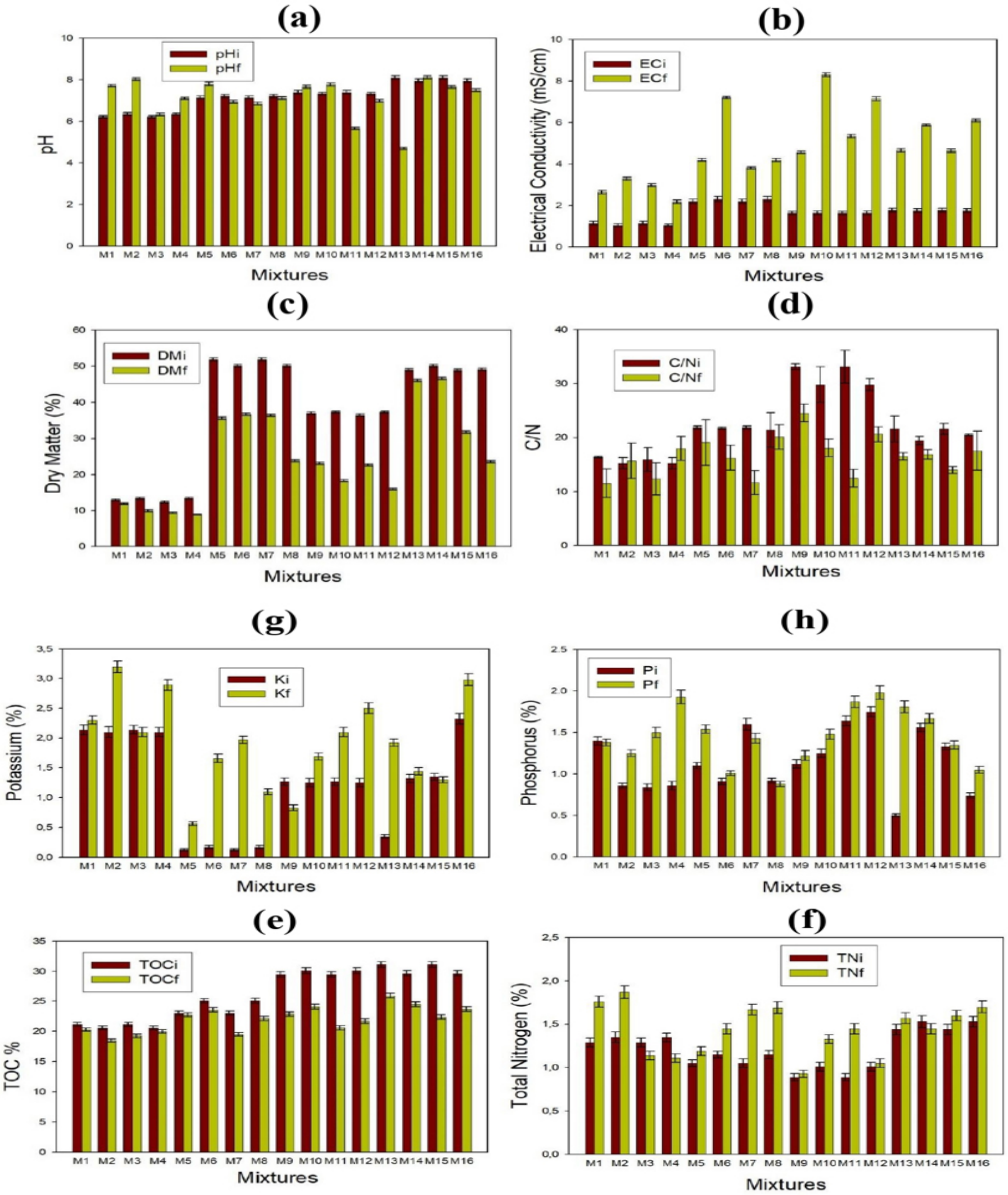

Figure 5. Evolution of physicochemical and nutritional parameters of 16 Mixtures in AD (a) Evolution of pH (b) Electrical Conductivity (c) Dry Matter (d) C/N ratio (e) Total Organic Carbon (f) Nitrogen (g) Phosphorus (e) Potassium.

as co-substrate but no fungal inoculation presented the lowest biogas yield, which reflects the negligible effect of this co-substrate even if it seems to be balancing the C/N ratio, its effectiveness does not appear to be significantly influencing the mixture unless it is combined with fungal inoculation, as molasses is considered as carbon-rich substrate which results in an adequate source of carbon for *A. niger* that helps to improve the microbial hydrolysis.

The Figure 4b shows the evolution of the mixture's mass over 21 days of AD. We can observe from the figure that the weight loss follows the same trend as biogas production. It actually decreases gradually, following a kinetic trend similar to that of biogas production. This consistency reflects a distinct proportionality between the level of biogas generated and the degree of substrate degradation. For instance, M12, M16, and M2 were high-yield mixtures, and consequently, they experienced the largest mass reduction. Meanwhile, M1, M9, and M11 presented the smallest mass losses, and simultaneously, they were low-yield mixtures. These findings demonstrate that greater bioconversion efficiency directly results in more weight loss and the breakdown of volatile solids (Nayeri et al., 2024).

Evolution of Physicochemical and Nutritional Parameters

Figure 5a shows the pH evolution kinetics for 16 mixtures between the initial and final day. The pH evolution reflects the microbial activity and gives an idea about the process stability. Overall, an upward pH shift indicates balanced acidogenesis and methanogenesis, with a pH range moving from 6.8 to 8.2, as methanogens convert acidogenic intermediates, favoring slightly alkaline conditions (Appels et al., 2008).

The pH increase remains a good indicator of the balanced acidogenesis and methanogenesis of the AD process. M1 (7.71), M2 (8.03), and M14 (8.11) have all experienced an increase in their final pH, which reflects an efficient buffering capacity. In fact, these mixtures were also considered as top-performing mixtures in terms of biogas yield, and these pH values are within the ideal range for microbial consortia that are responsible for producing biogas. M3, which had a pH shifting from 6.21 to 6.33, actually shows a stagnant pH evolution, indicating acid buildup and a low volatile fatty acids conversion. This limited methanogenic activity in M3 explains the low biogas output obtained for this mixture.

M13 showed a significant pH drop observed in M13 from (8.1 to 4.7) and M1 (7.4 to 5.66). This acidification is likely caused by excessive hydrolysis or unbalanced substrate composition, which results in the inhibition of the methanogenic activity and consequently gives a low biogas yield (López et al., 2020). M12, which showed a slight decline in pH (7.33 to 6.98), remains within the acceptable methanogenic range. The important buffering capacity in M12 limited acid accumulation despite its pH decline, which is why this mixture was also a good biogas producer even within a slight pH decrease.

M15 and M16 maintained a considerably stable pH value after digestion, as the final pH falls within the ideal methanogenic window (7.0-8.0). The stability in these mixtures is purely linked to their steady and high methane yields. Both mixtures were under thermophilic conditions and contained molasses as a co-substrate; these conditions may be responsible for the enhancement of pH stability and biogas production performance. Additionally, M16 was inoculated with *A. niger*, which further improved the AD stability and methane production. The pH evolution across all mixtures is an important indicator of the synergic effect between substrate composition and microbial community, which leads eventually to a high bioconversion efficiency. Mixtures that showed a higher biogas yield and more efficient AD were all able to maintain pH within the optimal range. This correlation is also confirmed by the study of Meegoda et al (Meegoda *et al*., 2018). Meanwhile, the deviation from this range, especially acidification, can cause inhibition of methane production.

The electrical conductivity shows the level of mineral richness for each mixture before and after the end of the methanization process. Figure 5b shows the electrical conductivity variations across 16 mixtures during AD. It shows the amount of nutrient solubilization and ionic release during the process, which also gives an idea about the digestion efficiency, as the increase in this parameter is a major indicator of the mineralization process (Marín-Peña *et al*., 2020). The anaerobic degradation of proteins and components that are rich in nitrogen in poultry waste releases soluble salts such as potassium, phosphate, and ammonium ions, which explains why most mixtures experienced a clear rise in electrical conductivity between the starting and the end of the process.

The most notable increases were shown by mixtures like M10 (1.64 to 8.3 mS/cm), M14 (1.75 to 5.88 mS/cm), M6 (2.31 to 7.2 mS/cm), and M12 (1.64 to 7.14 mS/cm), indicating efficient substrate degradation and metabolite release. These results are consistent with the level of biogas productivity for these same mixtures, which refers to the strong link between the enhanced ionic mobility in the mixtures and the efficient bioconversion process. The rise in conductivity reflects active hydrolysis and acidogenesis phases, as during these stages, ammonium and solubilized organic compounds are released (Anukam et al., 2019). Mutually, a lower biogas yield is correlated with a moderated increase in electrical conductivity, and this was proven through M3, M4, and M1. The results shown for these mixtures can be explained by a partial substrate degradation or microbial inhibition, which causes a limited solubilization. However, even with their moderate-to-low biogas output, M13 and M11 exhibit significant EC increases. This could be because of the buildup of inhibitory ions (like ammonia or volatile fatty acids), which can lower methanogenic efficiency without necessarily limiting solubilization (Franke-Whittle et al., 2014). Overall, conductivity variations are indicators of the level of mineral release and solubilization efficiency, but they also reflect the potential toxic thresholds or imbalances that could influence microbial activity.

Dry matter is one of the key indicators of the efficiency of AD and the degradation of substrates. Figure 5c illustrates that there was a progressive decrease from the first day to the last for all mixtures. This reduction is an important confirmation that microbial consumption of the organic compounds happened through their digestive process. The dry matter results are scattered among all mixtures, which signifies that all mixtures demonstrated different levels of biodegradability, microbial activity, and substrate composition. For example, M12, M8, M10, and M16 have an important dry matter reduction with various initial values. In fact, high dry matter degradation is indicative of a strong biodegradability of substrates combined. Furthermore, these significant losses in dry matter correspond with high production of biogas in those digestates, leading to the idea that a higher consumption of organic matter is causing a higher biogas production. Fuess et al., in their study, have shown the effect of thermophilic conditions and co-digestion with molasses and their effect on microbial hydrolysis and methanogenesis, and these mixtures were all inoculated with *A. niger*, and four of them either had thermophilic conditions or were formulated with molasses (Fuess *et al*.,2021). M5, M6, M7, M9, M11, and M15 showed a moderate decrease in dry matter. This reduction means that the substrate conversion was slightly lower, which is possibly caused by a suboptimal C/N ratio, low moisture, or slow microbial activity. We can also include the role of moisture in here, as the mixtures that are under solid-state conditions may limit mass transfer and also cause poor enzyme diffusion if the digestate is not hydrated enough, resulting in slow and inefficient degradation. These findings have been confirmed by Veluchamy and Kalamdhad (2017) in their study, suggesting that high-solid AD can limit substrate availability and reduce hydrolysis rate. We also observe a limited dry matter loss in M1, M2, M3, M4, M13, and M14, suggesting a possible low biodegradability or even a microbial inhibition. M13, as an example, experienced only a minimal decrease, which can be linked to its very low final pH.

Similarly, mixtures M1 and M3, which had low initial dry matter, presented minor reduction, following the very low biogas yield, probably because of the insufficient microbial colonization or even nutrient availability. This consistent reduction across all mixtures, especially when this corresponds to biogas production, strengthens the principle of substrate-to-gas conversion in the AD system. That shows that moisture content is also considered as an important influential factor in the process (Wang et al., 2023).

The C/N ratio is a major indicator of microbial and chemical stability of the final digestate and AD performance. This ratio must generally be between 15 and 30 to be considered suitable for microbial activity and digestate maturity (Wang et al., 2023).

As shown in Figure 5d, mixtures that start with a balanced or low C/N ratio, such as M2 (15.23), M12 (29.81), and M16 (20.48), showed higher biogas production, reaching 14,000-16,000 mL at the end of digestion, which explains the importance of molasses introduction into the anaerobic system as an important substrate (Fuess et al., 2024). These initial ratios provide a favorable environment for the methanogenic consortium, ensuring good carbon availability for the energy of microorganisms, specifically *A. niger*, while avoiding the toxic effects that can be caused by excess nitrogen, such as ammonia inhibition. The final values of these mixtures fall within the optimal range for good microbial activity (M2: 15.7, M12: 20.63, M16: 17.55), indicating efficient use of substrates and well-advanced, stable digestion, specifically with M12 marking the highest biogas yield actually has a C/N ratio of 20.63. This result is strongly in alignment with what Ahmad et al. (2024) found in their study, indicating that the highest specific biogas yields was when the feedstock had a C/N ratio of 20, surpassing yields at higher values. In addition, mixtures M1, M3, and M11, with a significantly low C/N ratio for all three mixtures, produced a low total volume of biogas, probably due to unbalanced stoichiometry or excessive ammonification interfering with microbial enzymes.

Mixtures with a stagnant or slightly decreasing C/N ratio (M5 and M13) are also associated with low biogas production, proving the minimal existence of nutrients, which remain insufficient. This confirms results of previous mixtures where maintaining the C/N ratio between 20 and 30 is optimal for anaerobic systems in order to avoid excessive acidification and ammonia toxicity (Wang et al., 2023). Therefore, the C/N ratio not only explains the variability in biogas production across different mixtures but also serves as a chemical fingerprint for process stability and conversion efficiency, proving that biogas production is chemically linked to the balanced stoichiometry between degradable carbon and available nitrogen.

Figure 5e shows the total organic carbon values for 16 mixtures during AD. These values reflect the microbial degradation of organic carbon during the process into biogas components, particularly methane (CH_4) and carbon dioxide (CO_2). Mixtures M2, M3, M6, M7, M8, M9, M10, M11, M12, M15, and M16 experienced a significant reduction in total organic carbon, with the largest reduction observed for mixture M12, ranging from 31.11% to 21.66%, which logically explains its intense biogas production, caused by the effective degradation of carbonaceous materials. In addition, M1, M4, and M5 showed the lowest decrease in TOC percentage and similarly low biogas production (M1 being the lowest mixture in terms of biogas production), which can be explained by incomplete degradation of the substrates, microbial inhibition (accumulation of volatile fatty acids or ammonia stress) or non-biodegradable carbon fractions resistant to anaerobic conversion (Franke-Whittle *et al*., 2014).

The results on the evolution of total nitrogen in the 16 mixtures show different behaviors depending on the case as shown in Figure 5f. Some mixtures, such as M1, M6, M7, M8, M13, M14, and M15, had an increase in their nitrogen levels, while others, such as M2, M3, M4, and M9, showed a slight decrease or remained stable. These changes can be explained by two phenomena: either the nitrogen was released (mineralization), or it was used by microorganisms or lost in the form of gas (Mahmud et al., 2021). When organic matter is well degraded, especially in humid and stable environments such as M1 or M8, microorganisms convert the nitrogen in proteins into a form that can be used by plants (nitrate and ammonium). This often goes hand in hand with good biogas production (Deng *et al*., 2025).

However, in mixtures such as M4 or M9, there is not much change, probably because *A. niger* and the other microorganisms have used the nitrogen to construct their cells or because the nitrogen has been released as a gas due to high pH or high temperature. In the case of mixture M13, where the pH is very acidic, the organisms may be under stress and not be able to break down the proteins properly (Duong et al., 2019).

Overall, these nitrogen results confirm what we discussed earlier regarding the biogas production, pH, dry matter, and C/N ratio. A good mixture produces more biogas, remains safe at the microbial level, and retains more elements that are useful for plants. This shows that nitrogen is a good indicator of whether AD is successful and whether the final digestate is considered a good fertilizer.

As shown in Figure 5g, the amount of phosphorus increased in almost all mixtures during AD. For example, M4 showed a significant increase along with M13. These changes reflect the mineralization of organic phosphorus and its transformation into mineral phosphorus, which obviously aligns with the degradation of organic compounds and the release of ionic components. This mineralization is often accelerated by enzymatic activity in the presence of *A. niger*, which has been proven by several studies. for instance, Tian et al. (2021) demonstrated in their study that this fungus has a considerable ability to solubilize phosphorus through its acidifying potential and the secretion of acids, especially oxalic acid and citric acid. The degradation of phytate compounds and the lysis of microbial cells during fermentation also contribute to the release of soluble phosphate. It is important to note that this phosphorus mineralization is extremely important for the production of a nutrient-rich digestate suitable to be used as a biofertilizer (Chen et al., 2024).

Surprisingly, mixtures M3, M7, and M15 revealed minor changes. This may indicate either low microbial transformation or low availability of phosphorus in organic forms or sorption onto solids, especially in solid fermentation mixtures. This can limit the diffusive process of chemical elements (Mazzini et al., 2020).

In Figure 5h, potassium showed more variable changes. For example, M2 increased from 2.1 to 3.2, and M16 increased from 2.32 to 2.98, suggesting effective solubilization of potassium from the biomass during degradation. In contrast, M3, M9, and M15 experienced a decrease in potassium levels, which can be explained by absorption into the microbial biomass or binding to organic residues (Wang & Huang, 2001). Potassium is a soluble ion that usually can be leached early in the process, particularly in liquid fermentation. High final concentrations of potassium, though, could be a result of high levels of moisture or well-degraded biomass. But additionally, low mobilization could result from low water activity. (Oriol et al., 1998).

The phosphorus and potassium data indicate that these nutrients are released from organic material during AD and can serve as proxies for complete degradation. Their final concentrations, especially with their results, are based on pH value, C/N ratio, and biogas yield. These indicators can be used to estimate both methanogenic activity and the nutrient potential of the digestate. The most efficient mixtures regarding biogas productivity also included high mineralization, thus ranking them amongst the best mixtures to recover energy, and also with a high biofertilization potential.

Microbiological analysis and determination of ions and heavy metals

Table 3 shows the microbiological analyses according to NF U 44-051:2006 for *E. coli* and *Salmonella*.

We observe that M1, M5, M7, M9, M11, M13, and M15 were all considered non-compliant mixtures, as their final results showed incomplete inactivation of at least one of the two pathogens on the final day of AD. Meanwhile, M12, which is top-performing in biogas yield, actually achieved a complete microbiological safety on the final stage of the process, which indicates that the high microbial activity in this digestate and also the thermophilic conditions led to an effective pathogen destruction. M16, which is another top performer in biogas production and hygienic status, was also characterized by a thermophilic AD, inoculation with *A. niger*, and molasses co-digestion, which collectively enhanced

Table 3. Analysis of microbiological safety criteria of the 16 studied mixtures.

	E.coli		***Salmonelle***	
NF U 44–051 : 2006 Standards	<100 UFC/g		Not detected in 25g	
Mixtures	**Ti**	**Tf**	**Ti**	**Tf**
M1	NC	NC	C	C
M2	C	C	C	C
M3	NC	NC	C	C
M4	NC	C	C	C
M5	NC	NC	NC	C
M6	NC	C	NC	C
M7	NC	C	NC	NC
M8	NC	C	NC	C
M9	NC	NC	NC	C
M10	C	C	NC	C
M11	NC	C	NC	NC
M12	NC	C	NC	C
M13	NC	NC	C	NC
M14	NC	C	C	C
M15	NC	NC	C	NC
M16	C	C	C	C

Table 4. Heavy metals results according to NF U 44-051 standard

Mixture	**Cu**	**Ni**	**Pb**	**Cd**
NFU 44-051	< 300	< 60	< 180	< 3
M1	125.0 ± 4.01*	36.0 ± 2.09*	154.0 ± 5.3*	0.20 ± 0.00*
M2	102.0 ± 3.2*	55.0 ± 3.01*	130.0 ± 4.0*	0.05 ± 0.00*
M3	201.0 ± 6.04*	26.0 ± 2.03*	121.0 ± 4.0*	0.07 ± 0.00*
M4	145.0 ± 4.1*	18.0 ± 1.0*	104.0 ± 3.0*	0.21 ± 0.00*
M5	216.0 ± 7.2*	15.0 ± 1.5*	65.0 ± 3.0*	0.65 ± 0.00*
M6	133.0 ± 4.03*	31.0 ± 2.2*	88.0 ± 3.0*	0.10 ± 0.00*
M7	87.0 ± 3.06*	54.0 ± 3.1*	65.0 ± 3.0*	0.09 ± 0.00*
M8	114.0 ± 3.01*	32.0 ± 2.02*	49.0 ± 2.0*	0.14 ± 0.00*
M9	115.0 ± 3.2*	42.0 ± 2.04*	124.0 ± 4.0*	0.09 ± 0.00*
M10	111.0 ± 3.04*	11.0 ± 1.01*	102.0 ± 3.0*	0.26 ± 0.00*
M11	265.0 ± 2,3*	21.0 ± 2.1*	152.0 ± 5.0*	0.34 ± 0.00*
M12	106.0 ± 3.2*	25.0 ± 2.3*	96.0 ± 3.0*	0.38 ± 0.00*
M13	166.0 ± 3.0*	41.0 ± 2.2*	54.0 ± 2.0*	0.05 ± 0.00*
M14	244.0 ± 4.1*	49.0 ± 3.0*	34.0 ± 2.0*	0.71 ± 0.00*
M15	154.0 ± 4.05*	44.0 ± 2.03*	141.0 ± 5.0*	0.41 ± 0.00*
M16	209.0 ± 5.2*	39.0 ± 2.04*	93.0 ± 3.0*	0.05 ± 0.00*

*: Conform to the threshold value

the antimicrobial potential. This fungus who was present in all five of the best-compliant mixtures (M2, M4, M10, M12, and M16), is known for its pathogen-lysis ability through the release of secondary metabolites, while the high temperature can cause an important damage to these pathogens by accelerating enzymatic hydrolysis and denaturing their membrane (Seruga et al., 2020; Yu et al., 2021). The pH is also an important factor in the hygienic safety of the mixtures. For instance, M13 was found to be extremely acidic (pH 4,7), and this level of acidity may encourage the persistence of *E. coli* but not that of methanogens (Pandey et al., 2011). On the other hand, M16 has maintained a stable pH, which optimally supported the high biogas yield and the efficient pathogen lysis. These findings are consistent with the study of Seruga et al. (2020) which indicates that more stable pH enhances the process effectiveness and pathogen suppression.

Tale 4. Shows the final results of heavy metals for all 16 mixtures. The analyzed were found to comply with heavy metal standards in accordance with NF U 44-051, confirming their agronomic safety for agricultural use. Concentrations of copper, nickel, lead, and cadmium are within acceptable limits, although some mixtures, such as M11 and M15, are close to the limits for copper and cadmium; they remain acceptable. The presence of *A. niger* and high organic matter often led to metal immobilization through biosorption and chelation mechanisms, while the solid matrix and microbial activity may have contributed to metal stabilization. Overall, these results once again demonstrate the environmental safety and potential for effective use of digestates in the waste recovery process (Kapoor & Viraraghavan, 1997).

Summary of nutritional analyses in terms of the NF U 44-051 standard

The 16 AD mixtures were evaluated according to standard NF U 44-051 as shown in **Table 5**, which requires a minimum Total Organic Carbon (TOC) content of 20% and maximum limits of 3% for Total Nitrogen (TN), Phosphorus (P), and Potassium (K). According to the mentioned standards, M2, M3, and M7 were rejected because of their low Total organic carbon values (below 20%). Among the remaining mixtures, M4, M12, and M16 presented respectively the highest NPK values (6.75%, 6.38%, 5.38%), noting that M4 is the richest in total nutrients and M16 is the highest in potassium content, however, when taking into consideration the C/N ratio who is identified as key indicator for the maturity of the compost and nitrogen availability.

Table 5. Nutritional analysis in terms of the NFU 44-051 standard

	TOC (%)	**N (%)**	**P (%)**	**K (%)**
M1	20.25 ± 0.28	1.76 ± 0.06	1.38 ± 0.04	2.30 ± 0.07
M2	18.44 ± 0.27 ^{nc}	1.87 ± 0.07	1.25 ± 0.04	3.20 ± 0.10
M3	19.25 ± 0.29 ^{nc}	1.14 ± 0.05	1.50 ± 0.06	2.10 ± 0.08
M4	19.96 ± 0.30 ^{nc}	1.11 ± 0.05	1.93 ± 0.08	2.89 ± 0.09
M5	22.72 ± 0.33	1.19 ± 0.05	1.54 ± 0.05	0.57 ± 0.03
M6	23.56 ± 0.35	1.45 ± 0.06	1.01 ± 0.03	1.66 ± 0.07
M7	19.50 ± 0.31 ^{nc}	1.67 ± 0.06	1.43 ± 0.06	1.97 ± 0.06
M8	22.11 ± 0.36	1.69 ± 0.07	0.88 ± 0.03	1.10 ± 0.05
M9	22.84 ± 0.38	0.93 ± 0.04	1.22 ± 0.06	0.83 ± 0.05
M10	24.06 ± 0.40	1.33 ± 0.05	1.48 ± 0.06	1.69 ± 0.06
M11	20.59 ± 0.36	1.45 ± 0.06	1.87 ± 0.07	2.10 ± 0.08
M12	21.66 ± 0.38	1.05 ± 0.05	1.98 ± 0.08	2.50 ± 0.09
M13	25.96 ± 0.43	1.57 ± 0.06	1.81 ± 0.07	1.92 ± 0.06
M14	24.48 ± 0.39	1.45 ± 0.06	1.67 ± 0.06	1.44 ± 0.06
M15	22.36 ± 0.38	1.60 ± 0.06	1.35 ± 0.05	1.30 ± 0.05
M16	23.69 ± 0.40	1.70 ± 0.07	1.05 ± 0.04	2.98 ± 0.10
NF U 44-051	≥ 20%	< 3%	< 3%	< 3%

nc: Non-conforming to the standard

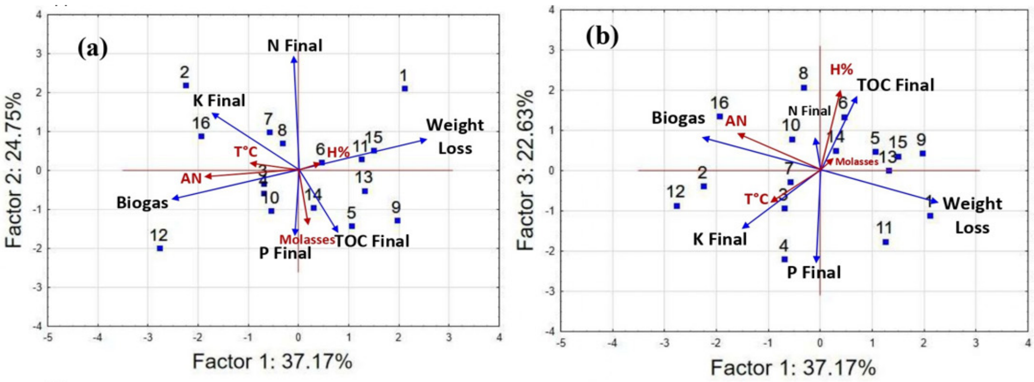

Figure 6. Principal Component Analysis PCA (a) Biplot represented by factor 1 (37.17%) and factor 2 (24.7%); (b) Biplot represented by factor 1 (37.17%) and factor 3 (22.36%**)**

The M12 mixture has the best C/N ratio (20.63), higher than that of M4 (17.98) and M16 (17.55), making it the most balanced option. A C/N ratio close to 20 is ideal for good microbial activity and for limiting nitrogen losses in gaseous or blocked form. Thus, even though M4 contains slightly more nutrients, the M12 mixture was chosen as the best candidate for biofertilizer production, thanks to its good balance between nutrient richness, regulatory compliance, and optimal C/N ratio for sustainable recovery.

Statistical analysis

From the principal component analysis (PCA) plots, according to **Figure 6**a represented by factor 1 (37.17%) and factor 2 (24.7%), and Figure 6b represented by factor 1 (37.14%) and factor 3 (22.63%), we can conclude several observations regarding the different interactions between the factors and the parameters of the mixtures. For example, the analysis shows that *A. niger* has a strong positive correlation with the biogas production axis and with less intensity with final potassium content, as well as a less significant positive correlation with the final phosphorus content, which explains the significant advantage of using inoculum such as *A. niger*, which makes the mineralization process more efficient (Doublali et al., 2025). Molasses supplementation is positively correlated with TOC content, which proves the effect of this factor on carbon input. This seems obvious, since the ultimate goal of adding molasses is to increase the carbon content in the medium, which indirectly influences the efficiency of mineralization by balancing the C/N ratio. Temperature also appears to contribute positively to the final potassium content, and also to the final phosphorus content, even if less significantly, and this can be explained by the fact that the solubility, availability and release dynamics of these elements can be directly affected by the increase in temperature through the improvement of enzymatic hydrolysis by microorganisms and the reduction of the adsorption of these elements to macromolecules (Kumar et al., 2024). However, the weaker positive correlation between temperature and phosphorus compared to potassium can be explained by the fact that phosphorus may still be subject to precipitation even after being released in ionic form. Chan et al note that even after phosphorus is released during AD, it can precipitate again in the form of mineral phosphate (e.g., with Ca, Mg, Fe) under certain pH and ionic conditions (Xi et al., 2023).

On the other hand, it can be noticed that the humidity axis is positively correlated with the total organic carbon axis, which explains the significant effect observed of high humidity on slowing down or inhibiting the degradation of organic matter and biogas production. These results are consistent with those found in a study by Hernandez-Berriel et al. (2008) which found that bioreactors maintained at 80% humidity had a lower methane production rate than bioreactors maintained at 70% humidity. Interestingly, we found that the M16, M2, and M12 mixtures seem to be particularly favorable, showing the strongest positive correlation with phosphorus, potassium, and biogas, and a relatively weaker correlation with nitrogen, which is also considered important for the final product. This is in line with previous results and interpretations, which reflect not only the efficiency of these mixtures in terms of biogas production, but also their high nutrient content, which makes them suitable for use as biofertilizers thanks to their balanced C/N ratio. The Pareto analysis reveals that biogas production was the most influenced parameter, with a significant effect of *A. niger* exceeding the statistical threshold (p-value line), confirming the results previously obtained on the effect of this fungal species on biogas production. Therefore, even if the samples number is quite low, it is evidently observed that each of these factors used in the study has a very important effect on the parameters studied, for instance, *A. niger* affects the biogas production directly, The Addition of molasses influences the TOC initial input which balances the C/N ratio, The temperature affects the potassium levels directly, which means that if all these factors are combined with the right percentages they can improve the AD process from both perspectives; biogas production and biofertilization potential (**Figure 7**).

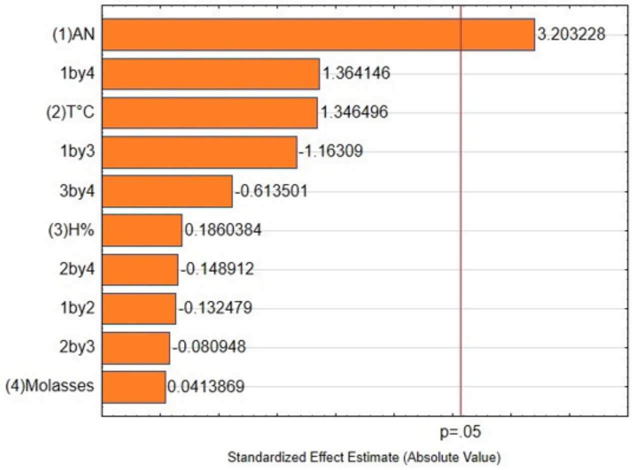

Figure 7. Pareto diagram representing the statistical significance of the effect of the four factors

Germination test

Digestate M12 showed no inhibitory effect on tomato seed germination during the phytotoxicity test, proving that the mixture did not develop any toxic or growth-inhibiting molecules during the AD process, as the germination rate was 80% over a period of 170 hours. This absence of phytotoxicity proves that harmful intermediates such as volatile fatty acids, phenolic compounds, or free ammonia, which are in some cases produced under suboptimal conditions of the AD, were either not produced or were completely degraded during the process. Furthermore, this proves its safety, specifically in terms of heavy metals. As a result, digestate M12 is suitable for a fertilization test.

Fertilization test

The fertilizer characteristics and fertilization potential test are shown in **Table 6**. The test indicates that M12 liquid fraction is the best option for growing tomato crops. Additionally, because of its high potassium content, which plays an important role in root elongation and water regulation, M12 liquid-fraction performs better in vegetative growth, producing a more resilient plant that is better able to adjust to changes in the environment and nutrition. Additionally, the important percentage of phosphorus in M2 digestate supports leaf formation and energy transfer (Sustr *et al*., 2019; Khan *et al*., 2023). The optimal C/N ratio for M12 helped remarkably in the stable release of nitrogen without any ammonium accumulation. Even if this element was average in the digestate, its continuous availability was an important support in stem growth and thickness equally.

The liquid fraction of M2 presented an important advantage over the solid fraction, thanks to the availability of nutrients and the faster absorption, resulting in a better vegetative support. The average number of leaves was also more important for the M12 fraction, which is a key indicator of vegetative vigor, indicating the nutrient balance and availability that this fraction actually has, making it a very effective biofertilizer.

In terms of chlorophyll a, chlorophyll b, and carotenoids, the liquid fraction of M12 presented the highest results, surpassing the control and solid fraction, and slightly matching the commercial fertilizer. These levels are a result of the good C/N ratio balance, as well as the effective nitrogen mineralization through the elevated total organic carbon levels that improved microbial activity, which resulted eventually in good nitrogen levels that support chlorophyll synthesis (Fathi, 2022; Reuland et al., 2022). The moderate levels of phosphorus in the digestate contributed to ATP production, resulting in a better energy transfer, while the high amount of potassium regulated stomatal function and reduced oxidative stress, which enhances carotenoid formation (Carstensen et al., 2018; Hasanuzzaman et al., 2018). The highly rich agronomic profile of this M12 digestate was the main reason behind its superior pigment levels, which confirms once more the biofertilization potential of this digestate.

This visual comparison, shown in **Figure 8**, reflects the effect of different fertilizers on tomato root

Table 6. Comparative evaluation of fertilization potential for the M12 mixture

	Root Length (cm)	Stem		Leaf		Chlorophyll a	Chlorophyll b	Carotenoids
		Length (cm)	Thickness (cm)	Length (cm)	Average Amount	(mg g^{-1} FW (Fresh weight))		
Control	13.9 ± 0.04	7.1 ± 0.03	2.8 ± 0.01	4.0 ± 0.02	13.5 ± 0.06	8.00 ± 0.05	9.78 ± 0.05	7.20 ± 0.05
Commercial	17.8 ± 0.05	8.4 ± 0.04	3.2 ± 0.02	5.1 ± 0.03	15.25 ± 0.07	10.57 ± 0.06	13.48 ± 0.09	11.04 ± 0.08
M12 (Solid Fraction)	14.3 ± 0.04	7.3 ± 0.03	3.4 ± 0.02	4.5 ± 0.02	16.0 ± 0.06	8.80 ± 0.04	12.56 ± 0.06	7.18 ± 0.01
M12 (Liquid Fraction)	17.1 ± 0.05	7.9 ± 0.04	3.7 ± 0.01	4.8 ± 0.01	18 ± 0.05	10.58 ± 0.05	12.5 ± 0.06	10.59 ± 0.02

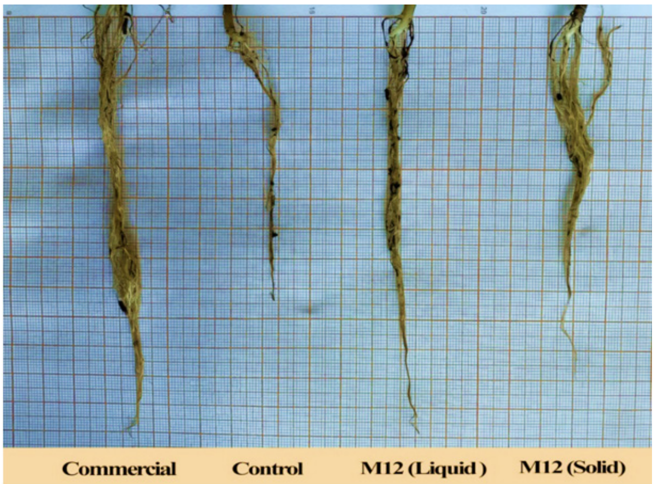

Figure 8. Visual Comparison of Tomato Root Development

development. The roots from plants treated with the liquid fraction of M12 appear to be longer and more uniform than those of the control or even M12 with its solid fraction, and closely approach the roots treated with commercial fertilizer in terms of length and density. The commercial fertilizer results in the most robust root system, followed by the liquid fraction of the M12 mixture, confirming previous measurements. This supports previous results showing that the high potassium content and favorable nutrient profile contribute significantly to improving root structure and elongation. The solid fraction of M12 also showed promising results, outperforming the control. Overall, the results confirm the potential of M12, especially the liquid fraction, as a promising biofertilizer.

Conclusion

In order to minimize, even to a small percentage, the severely damaging impacts caused by this waste, Morocco's major challenge of managing poultry waste has long required immediate government action through the control and regulation of this sector. However, the task is always made more difficult due to the lack of resources and professional channels that specialize in the treatment and recovery of waste, particularly given the variety of production sources and the demographic development that undoubtedly comes with an increase in meat consumption and, consequently, an increase in the rate of poultry waste.

This work shows that it is strongly possible to find an effective solution to this problem through the adoption of a purely ecological approach, by the valorization of poultry waste to produce biogas and biofertilizers that are potentially effective for energy generation and plant crops.

This study has shown the effect of the factorial design 2^4 and the combination of different factors on biogas production and the content of macronutrients and minerals, in such a way that the same waste source, which is poultry waste with same quantity found in the sixteen mixtures do not give similar results in terms of biogas potential and nutrient parameters, and this is due to the synergic effect of combining the four different factors involved in this study, especially *A. niger* who has improved biogas production remarkably. The best mixture to produce a potentially effective biofertilizer for plant crops was the same best-performing mixture in biogas production levels with more than 17.000 mL of biogas produced over 21 days, which is the M12 digestate. This mixture had an important amount of nutrients (N (1.45%), P (1.98%) and K (2.5%)) and a suitable C/N ratio (20.63), and it was processed under thermophilic conditions with fungal inoculation and the presence of molasses, and all these factors were found to positively affect the nutritional parameters and biogas yield.

This innovative strategy is strongly consistent with ecological principles, which require that materials be recycled while utilizing their benefits and protecting our natural resources and, consequently, our planet.

Declaration of competing interest

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

Acknowledgments

The authors would like to thank CNRST for its financial support of PPR2/2016/21 and Research Center of Materials and Energy of the Hassan 2 University of Casablanca

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.