Vol. 25, No. 2(2026) Bio26755

Revista Mexicana de Ingeniería Química

Phytoregulators production through anaerobic digestion of dairy manure: evaluation of initial pH and digestion time effect

Producción de fitorreguladores a través de la digestión anaeróbica de estiércol de vaca: evaluación del efecto del pH inicial y el tiempo de digestión

M.M. Solís-Oba[1], J. J. Castro-Ramos[1,2]*, R. Castro-Rivera[1], A. Solís-Oba[3]

[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, México.

[2]Secretaría de Ciencias, Humanidades, Tecnología e Innovación, México.

[3]Universidad Autónoma Metropolitana unidad Xochimilco, México.

Received: January 12, 2026; Accepted: April 13, 2026

Abstract

Phytoregulators are widely used in agriculture to improve crop quality and yield. Currently, they are produced mainly through aerobic axenic fermentation; however, anaerobic digestion presents a potentially viable alternative, as it reduces energy requirements and allows the use of waste substrates. The effect of initial pH and digestion time on gibberellic acid (GA_3), indole-3-acetic acid (IAA), and abscisic acid (ABA) through anaerobic digestion (AD) of dairy manure (DM) was evaluated. A completely randomized factorial design was implemented using initial pH [5.5, 6.5 (control), 7.5, 8.5] and digestion time (0, 4, 8, 12, 16, 20 days) as factors. The ADs temperature was kept at 42 °C, the total solids was set at 7 %, and no external inoculum was added. Both main effects and their interaction showed a highly significant effect on GA_3 and IAA production. The highest overall GA_3 concentration (124.86 mg/L) was obtained at initial pH 8.5 and 8 days. IAA production occurs only in initial acid pH treatments, reaching a maximum concentration of 5.52 mg/L at initial pH 5.5 and 20 days. ABA was not detected under the evaluated conditions. The acidification behavior across all AD treatments suggests that GA_3 and IAA were produced during the hydrolytic-acidogenic stage of AD. Initial pH and digestion time highly influenced the profile and production of phytoregulators from DM via anaerobic digestion.

Keywords: anaerobic digestion, dairy manure, gibberellic acid, indole-3-acetic acid, phytoregulators.

Resumen

Los fitorreguladores son utilizados en la agricultura para mejorar la calidad y el rendimiento de los cultivos. Actualmente son producidos principalmente en cultivos axénicos aeróbicos; sin embargo, la digestión anaeróbica presenta una alternativa reduciendo requerimientos energéticos y utilizando sustratos residuales. Se evaluó el efecto del pH inicial y el tiempo de digestión en la producción de ácido giberélico (GA_3), ácido indol-3-acético (IAA) y ácido abscísico (ABA) en la digestión anaeróbica (DA) de estiércol de vaca. Se implementó un diseño completamente al azar con arreglo factorial utilizando el pH inicial [5.5, 6.5 (control), 7.5, 8.5] y el tiempo de digestión (0, 4, 8, 12, 16, 20 días) como factores. Las DAs se llevaron a cabo a 42 °C, con un contenido de solidos totales del 7 % y sin la adición de algún inoculo externo. Tanto los efectos principales como su interacción mostraron un efecto altamente significativo en la producción de GA_3 e IAA. La concentración más alta de GA_3 (124.86 mg/L) se obtuvo a pH inicial 8.5 y 8 días. El IAA se produjo únicamente en condiciones de pH inicial ácido, alcanzando una concentración máxima de 5.52 mg/L a pH inicial 5.5 y 20 días. No se detectó ABA bajo las condiciones evaluadas. La acidificación en todos los tratamientos de DA sugieren que el GA_3 y el IAA se produjeron durante la etapa hidrolítica-acidogénica.

Palabras clave: digestión anaeróbica, estiércol de vaca, ácido giberélico, ácido indolacético, fitorreguladores.

* *Corresponding author. E-mail*: jonathan_castro@live.com.mx ;
https://doi.org/10.24275/rmiq/Bio26755
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

The inadequate disposal of livestock waste, including dairy manure (DM), generates significant environmental impacts due to its high organic matter content, nutrients availability (nitrogen, phosphorus and potassium), and methane production potential (Glover *et al*., 2023; Liu *et al*., 2018). This contributes to soil and water pollution as well as greenhouse gas emissions (Font-Palma, 2019; Parihar *et al*., 2019). This issue is particularly concerning due to the intensification of livestock production to address the increasing global food demand (Soyer and Yilmaz, 2020). It is estimated that 3.4 billion dry metric tons of cattle manure are produced each year (Chuenchart *et al*., 2024).

These wastes could be revalorized through biotechnological processes offering a sustainable approach to transform them into valuable bioproducts; one example is the production of phytoregulators by anaerobic digestion. Phytoregulators are compounds produced by plants, microorganisms, or through chemical processes, which regulate physiological processes of plant development. Phytoregulators are used in agriculture to stimulate plant growth, improve crop yields and quality, and optimize management practices (Karishma *et al*., 2019).

For example, Islam *et al*. (2023) reported that foliage application of gibberellic acid (GA_3) at 200 ppm stimulated plant growth and increased the grain yield in mung bean crops. Nguyen *et al*. (2019) found that spraying GA_3 at 150 ppm increased the sugarcane yield by 19.94% and sucrose accumulation by 2.21%. Indoleacetic acid (IAA) application has been proven to mitigate cadmium-induced stress and improve growth and the physiological response in *Sorghum bicolor* (Bibi *et al*., 2025). IAA also was reported to improve selenium accumulation under stress conditions in grapevine; in addition, it increased the biomass of grapevine and the biomass of root and shoot by 15.61% and 23.95%, respectively, when applied at 60 ppm (Wang *et al*., 2024). Abscisic acid (ABA) has been used to mitigate the negative effects of drought stress. Zulfiqar *et al*. (2024) investigated the application of exogenous ABA at 100 and 200 ppm in wheat. They concluded that ABA improves drought resilience and the biochemical and quality characteristics of wheat, as well as increasing grain yield by 26.93% compared to the control. In high-density maize planting, the application of exogenous ABA improves grain filling and starch accumulation, thereby increasing grain weight and maize yield, as reported by Yu *et al*. (2024).

Gibberellic acid (GA_3), a phytoregulator from the gibberellins group, controls several plant development processes, including germination, flowering, leaf expansion, and stem elongation (Desai, 2017). Additionally, GA_3 plays an important role in increasing plant tolerance to abiotic stress (Emamverdian *et al*., 2020; Mahamud *et al*., 2025). Beyond agriculture, GA_3 is used in the malting of barley for the brewing industry (Siddikee *et al*., 2010), accounting for 17.2% of global market demand in 2016 (Grand View Research, 2022). Large-scale GA_3 production is carried out through aerobic submerged fermentation using the fungus *Fusarium fujikuroi* (Wang *et al*., 2022). Indole-3-acetic acid (IAA) is the most common auxin, participating in cell division, radicular expansion, tropisms, and apical dominance (Gutierrez *et al*., 2009). IAA is mainly produced by aerobic fermentation in axenic cultures using pure substrates (Shi *et al*., 2017). Abscisic acid (ABA) is a key phytoregulator involved in plant responses to both abiotic and biotic stress (Chen *et al*., 2020). Large-scale ABA production is obtained in fermentation processes by the fungus *Botrytis cinerea* (Wang *et al*., 2025).

Even though phytohormones are mainly produced via aerobic fermentation under controlled conditions (Shi *et al*., 2017), some investigations have revealed that they are also produced during the anaerobic digestion (AD) process (Castro-Sierra *et al*., 2024; Li *et al*., 2016). Li *et al*. (2016) explored the production of phytoregulators via the AD of dairy, swine, and chicken manures as substrates and sewage sludge as the source of inoculum. They observed the production of GA_3, IAA, and ABA throughout the AD processes. Castro-Sierra *et al*. (2024) reported the production of GA_3, ABA, and kinetin during the anaerobic digestion of bovine and porcine manure as substrates, concluding that the production of phytoregulators is influenced by the substrate and fermentation time. Despite these investigations, research exploring phytohormone production through AD remains limited.

Anaerobic digestion is a microbiological process in which diverse microorganisms degrade the organic matter in the absence of oxygen, producing biogas, a renewable energy, and digestate (Hernández-Eugenio *et al*., 2025; Solé-Bundó *et al*., 2017). AD comprises four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each involving distinct microbial communities with specific requirements for pH, temperature, and carbon to nitrogen (C:N) ratio (Alvarado-Reyna *et al*., 2024; Srisowmeya *et al*., 2020). Among the physical factors influencing AD performance, pH is one of the most critical, as it affects microbial growth, metabolism (Jin and Kirk, 2018), and the production of metabolites such as phytoregulators.

The production of phytoregulators via anaerobic digestion presents a promising alternative worth exploring. This approach offers multiple advantages, including waste valorization, lower energy consumption compared to aerobic processes, and the potential for the simultaneous production of various bioproducts and renewable energy (Almeida-Streitwieser, 2017). Moreover, it aligns with the principles of the circular economy. These advantages could make anaerobic digestion a competitive alternative to current production methods for plant hormones.

Although the effect of pH on other AD metabolites, such as volatile fatty acids, has been reported (Feng *et al.*, 2018), how pH influences phytoregulator production is not well established. Evaluating the effect of the initial pH is essential for understanding the anaerobic production of GA_3, IAA, and ABA. The aim of this study was to evaluate the initial pH and digestion time effect on phytoregulator indole acetic acid, abscisic acid, and gibberellic acid production through the anaerobic digestion process using DM. The present study contributes to our understanding of how operational variables, such as digestion time and initial pH, influence the production and profile of phytoregulators via anaerobic digestion of dairy manure. This knowledge can be used to design digestates as plant fertilizers with specific proportions of phytoregulators for specific applications.

2 Materials and methods

2.1 *DM characterization and preparation*

Fig. 1 depicts the general methodology scheme of the present study. The DM was obtained from the dairy bovine module at the Instituto Tecnológico del Altiplano de Tlaxcala in Mexico. The dairy cattle were fed with a standardized diet. The DM was obtained by composite sampling. Ten random subsamples of fresh DM were taken from individual cow feces and mixed.

DM was used both as a substrate and as inoculum for the anaerobic digestion processes evaluated in this study. The characterization of the DM included determination of pH, total solids (TS), volatile solids (VS), and the carbon-to-nitrogen ratio (C:N). The pH was measured using a Conductronic pH 120 device, after adjusting TS to 7% with deionized water (APHA, 2017). TS and VS were determined by drying the manure at 103°C and by incinerating at 550°C, respectively (APHA, 2017). The C:N ratio was determined using a CHNS-O Flash 2000 elemental analyzer, with helium as the carrier gas, following the manufacturer's instructions.

For anaerobic digestion, the total solids content of the DM was adjusted to 7% using deionized water, and the mixture was thoroughly homogenized under anaerobic conditions.

2.2 *Experimental design*

To assess the impact of initial pH and digestion time on GA_3, IAA, and ABA production during anaerobic digestion of DM, a completely randomized design with a factorial arrangement was used: initial pH values of 5.5, 6.5 (control treatment), 7.5, and 8.5; and digestion times of 0, 4, 8, 12, 16, and 20 days at 42°C. Three experimental replicates were used for each AD treatment.

2.3 *Anaerobic digestion*

Each of the anaerobic digestion treatments was performed in triplicate using 120 ml serological bottles, with an operational volume of 70 ml of the homogenized DM mixture (7% TS). The initial pH was adjusted to 5.5, 6.5, 7.5, and 8.5 using Na_2CO_3 or HCl, as per the experimental design. To maintain anaerobic conditions, nitrogen gas was introduced into the headspace of each serological bottle to displace air. The digesters were then incubated at 42°C in a controlled environment using a Felisa FE-131A incubator, with no agitation. Three serological bottles were taken out at the specified digestion times (0, 4, 8, 12, 16, and 20 days) to analyze phytoregulators (IAA, GA_3, and ABA), and pH.

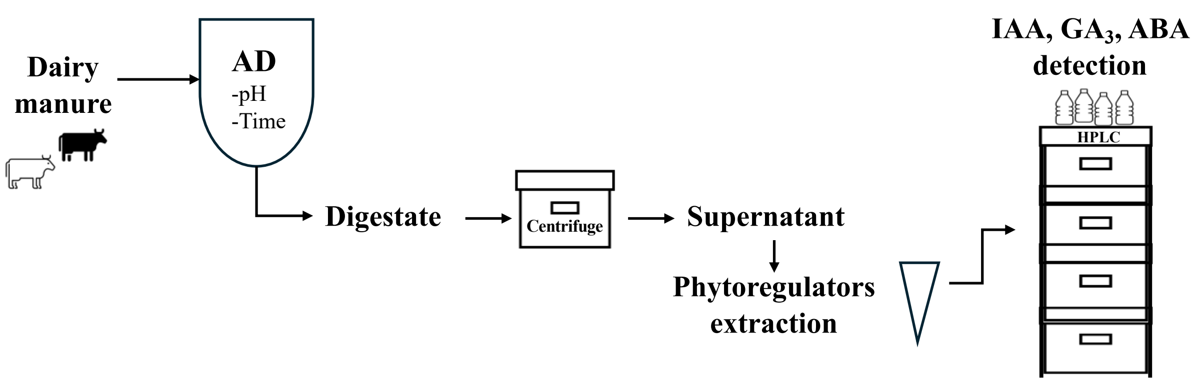

Fig. 1. General methodology scheme.

Table 1. Dairy manure characterization and reported requirements for the anaerobic digestion microorganisms.

Parameter	Value	Microorganism requirements (Deublein and Steinhauser, 2008) Hydrolytic- acidogenic	Methanogenic
Total solids (%)	14.00 ± 0.17	<40	<30
C/N ratio	18.24 ± 0.49	10-45	20-30
pH	6.55 ± 0.06	5.2-6.3	6.7-7.5

2.4 Phytoregulators

To quantify the phytoregulators production (IAA, GA_3, and ABA), digestate was centrifuged, supernatant was mixed with ethyl acetate, and the organic phase (extract) was resuspended in a water-methanol (50:50) solution (Castro-Ramos *et al.*, 2022). Separation and quantification of IAA, GA_3, and ABA were conducted using high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) with an Eclipse XDB-C18 analytical column (4.6 mm ID x 250 mm, 5 μm) coupled to a Zorbax Reliance Cartridge guard column (4.6 mm ID x 12.5 mm, 5 μm). The mobile phases consisted of water (A), methanol (B), and a water-methanol mixture with 0.2% acetic acid (C). Gradient elution started with a composition of 50% C and 50% A. Between 0 and 20 minutes, A decreased from 50% to 25%, while B increased from 0% to 25%. These proportions were then maintained until 27 minutes. The injection volume was 20 μL and the column temperature 25°C. The concentration of phytoregulators was determined using calibration curves for IAA (208 nm), GA_3 (215 nm), and ABA (208 nm) analytical standards (Sigma–Aldrich).

2.5 Statistical analysis

Initial pH and digestion time were considered as factors. The initial pH levels were 5.5, 6.5, 7.5, and 8.5, and the digestion time levels were 0, 4, 8, 12, 16, and 20 days. The response variables were the concentrations of GA_3, IAA, and ABA.

Two-way analysis of variance (ANOVA) was conducted to determine statistical differences, as well as the main and interaction effects of the evaluated factors on phytoregulators concentration. The least significant difference (LSD) post hoc test with a significance level of $p < 0.05$ was used to determine statistical differences among the treatments. All analyses were performed using RStudio (version 2025.09.0+387).

3 Results and discussion

3.1 DM characterization

The physicochemical characteristics of the substrate are one of the key factors influencing microbial growth and metabolite production in AD processes. The composition of the DM used in this study is presented in Table 1. The DM contained 14.00 ± 0.17% of TS, of which 84% were VS. Compared to the 22.41% and 21.69% TS reported by Castro-Sierra *et al.* (2024) and Li *et al.* (2016), respectively. The low TS in the present study could be attributed to the fresh condition of the samples. The DM pH was nearly neutral at 6.55 ± 0.06. This pH value is characteristic of fresh DM, whereas an alkaline pH (8.5–9.0) is characteristic of stored manure due to the conversion of nitrogen into ammonia (Dospatliev *et al.*, 2015).

The C:N ratio of DM was 18.24 ± 0.49. The C:N ratio is crucial for the growth and development of microorganisms because it determines the availability and balance of carbon and nitrogen for energy production and protein synthesis, respectively. In AD, the C:N ratio plays a key role. A higher C:N ratio can cause an overproduction of carboxylic acids, which inhibits methanogenesis. Conversely, a low C:N ratio can cause the accumulation of toxic ammonia for microorganisms (Raja-Ram *et al.*, 2022). The optimal C:N ratio for AD has been reported to be around 20-30 for the overall AD process (Surra *et al.*, 2019). However, each AD microbial consortium, namely the hydrolytic-acidogenic and the acetogenic-methanogenic, has specific requirements, as depicted in Table 1. According to the requirements listed by Deublein and Steinhauser (2008), the pH and C:N ratio of the DM used in the present study were favorable for promoting hydrolytic-acidogenic microorganisms.

3.2 pH changes in anaerobic digestion treatments

A pH decrease was observed in all digesters from the beginning until the 8th day (Fig. 2), and it remained almost unchanged until the 20th day (no statistical differences, $p < 0.01$), indicating that regarding the initial pH, the acidogenic step predominates.

The observed decrease in pH digestates across AD treatments indicated a condition known as anaerobic acidification. This phenomenon occurs when methanization is inhibited due to a high organic load substrate or an imbalance in microbial communities. Anaerobic acidification has been associated with the production of valuable metabolites such as short-chain fatty acids, lactic acid, and ethanol, with pH playing a key role in determining the final product profile (Darwin *et al.*, 2019; Pore *et al.*, 2016).

Table 2. GA_3 (mg/L) concentration measured in the anaerobic digestion treatments at 0, 4, 8, 12, 16, and 20 days, and initial pH 5.5, 6.5, 7.5, and 8.5.

Initial pH	Digestion time					
	0 d	4 d	8 d	12 d	16 d	20 d
pH 5.5	0.03±0.06 **j**	52.07±2.13 **fg**	29.95±2.45 **h**	36.09±2.77 **h**	42.04±0.38 **gh**	36.21±1.06 **h**
pH 6.5	0.04±0.07 **j**	15.66 ± 0.44 **i**	55.44±4.31 **fg**	62.36±9.25 **f**	65.35±1.82 **ef**	58.55±1.54 **f**
pH 7.5	0.04±0.08 **j**	58.36 ± 0.6 **f**	76.7±10.48 **e**	95.58±2.68 **cd**	95.91±5.98 **cd**	99.07±4.44 **bcd**
pH 8.5	0.03±0.06 **j**	94.31 ± 7.69 **d**	124.86±6.23 **a**	105.07±9.07 **bcd**	111.66 ± 5.55 **ab**	110.26±14.38 **abc**

Treatments with the same letter are not significantly different according to the multiple comparison LSD test ($p<0.05$). ± indicates the standard error of the mean of three replicates.

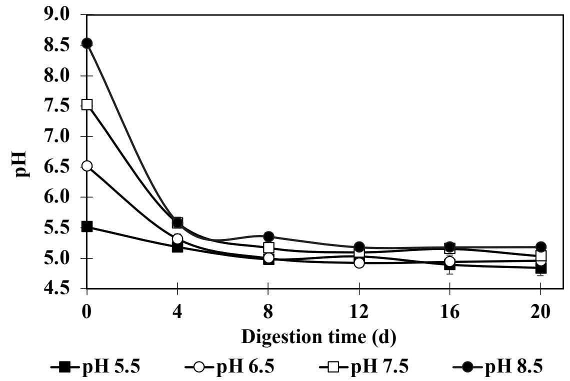

Fig. 2. Changes in digestate pH during the anaerobic digestion treatments.

The occurrence of anaerobic acidification in this study suggests that GA_3 and IAA were produced by hydrolytic-acidogenic microorganisms during the AD of DM. This is consistent with the findings of Li *et al*. (2016), who reported that GA_3 and IAA were generated during the hydrolysis–acidogenesis stage and remained nearly constant throughout the methanogenesis stage of AD.

The findings derived from the present study reveal the impact of initial pH modification on GA_3 and IAA production via anaerobic digestion. This could be attributed to the pH effect on the AD microbial community structure. Since AD involves a consortium of microorganisms with different pH preferences, initial pH can selectively stimulate or inhibit specific groups, altering organic matter degradation dynamics and, consequently, the metabolite profile. In this way, Liu *et al*. (2012) reported that the abundance of anaerobic digestion microorganisms is higher under neutral conditions than under acid or alkaline conditions. However, alkaline conditions promote the proliferation of hydrolytic microorganisms, while neutral and acid conditions favor acidogenic and acetogenic microorganisms (Liu *et al*., 2012; Ma *et al*., 2016).

3.3 Gibberellic acid production

Table 2 shows the GA_3 measured in each anaerobic digestion treatment. As initial pH increased, GA_3 production increased except for the AD treatments at 4 days. GA_3 production improved notably compared to the control treatment (initial pH 6.5), reaching a six-fold increase at initial pH 8.5 and 4 days.

On the other hand, no significant change in GA_3 production was observed between 12 and 20 days at any initial pH, suggesting that maximum GA_3 production can be obtained before 12 days regardless of initial pH under the evaluated conditions. The two-way ANOVA results indicated that both time and initial pH, as well as the interaction of these factors, had a highly significant effect on GA_3 production (Supplementary Table 1; Supplementary Fig. 1).

The highest overall GA_3 concentration (124.86 mg/L) was measured in the treatment at an initial pH of 8.5 and 8 days. In treatments at initial pH 5.5, 6.5, and 7.5, the maximum GA_3 production was observed at 4 days (52.07 mg/L), 16 days (65.35 mg/L), and 20 days (99.07 mg/L), respectively. In comparison, Li *et al*. (2016) reported a maximum GA_3 production of 47.18 mg/L at 14 days of anaerobic digestion of pig, dairy, and chicken manures.

The low GA_3 production and high energy consumption of conventional fermentation processes increase the price of the final product, limiting GA_3 extensive application in agriculture (Shi *et al*., 2016). Therefore, several investigations have explored different alternatives for GA_3 production, such as different microorganisms, culture systems, and substrates have been evaluated for GA_3 production. For example, Oliveira *et al.* (2017) evaluated GA_3 production by *Fusarium moniliforme* LPB03 and *Gibberella fujikuroi* LPB06 using submerged fermentation, solid and semisolid state fermentation, and citric pulp as substrate, reaching a maximum concentration of 273 mg/L. Rodrigues *et al*. (2009) evaluated the agro-industrial residues citric pulp, soy bran, sugarcane bagasse, soy husk, cassava bagasse, and coffee husk for GA_3 production in solid-state fermentation systems using *Gibberella fujikuroi* and *Fusarium moniliforme*, reporting a maximum production of 5.9 g/kg. Siddikee *et al*. (2010) achieved a GA_3 concentration of 400 mg/L using *Methylobacterium oryzae* under optimized conditions (30 °C, pH 7, C:N 5) in submerged, aerated axenic culture.

Table 3. IAA (mg/L) concentration measured in the anaerobic digestion treatments at 0, 4, 8, 12, 16, and 20 days, and initial pH 5.5, 6.5, 7.5, and 8.5.

Initial pH	Digestion time					
	0 d	4 d	8 d	12 d	16 d	20 d
pH 5.5	0.33±0.23 **e**	0.00	1.91±0.47 **cd**	2.42±1.26 **bc**	2.99±0.86 **b**	5.52±0.53 **a**
pH 6.5	0.4±0.26 **e**	3.44±0.39 **b**	0.00	0.16±0.03 **e**	0.66±0.1 **e**	0.75±0.07 **e**
pH 7.5	0.36± 0.01 **e**	0.00	0.00	0.00	0.00	0.97±0.01 **de**
pH 8.5	0.33±0.23 **e**	0.17±0.03 **e**	0.00	0.00	0.00	0.00

Treatments with the same letter are not significantly different according to the multiple comparison LSD test ($p<0.05$). ± indicates the standard error of the mean of three replicates.

Although in this study initial pH modification enhances GA_3 production compared to Li *et al.* (2016), it was lower than that obtained by Siddikee *et al.* (2010) under optimized aerobic conditions. Nevertheless, anaerobic digestion takes advantage of residual substrate utilization rather than pure substrates, requires no aeration or axenic culture, and has the potential for bioproduct and bioenergy co-production in a biorefinery context. Thus, AD is a promising and potentially cost-effective alternative for GA_3 production, especially if further optimization of operational parameters (C:N ratio, temperature, inoculum, substrate pretreatment) is pursued.

Conversely, since the concentration of GA_3 increased with the initial pH, exploring higher initial pH values would be an interesting study to investigate. Additionally, optimizing the AD process parameters could maximize both IAA and GA_3 production. Furthermore, IAA- and GA_3-enriched digestates produced through AD could be used as biostimulants in agriculture, eliminating the need for pure extracts.

3.4 Indole-3-acetic acid production

Table 3 shows the IAA production across the evaluated AD treatments. The multiple comparison test results indicated no significant differences between ADs at 0 days and the treatments at all the evaluated digestion times with initial pH values of 7.5 and 8.5, suggesting that no IAA was produced under these conditions (Table 3). In contrast, at the initial pH of 5.5, the IAA concentration increased with digestion time, obtaining the highest overall IAA production of 5.52 mg/L at 20 days. For treatments at initial pH 6.5, significant IAA production was observed only at 4 days, with a concentration of 3.44 mg/L (Table 3). These results suggest that IAA production is favored under acidic initial pH conditions.

The two-way ANOVA results revealed that digestion time, initial pH, and the interaction of these two factors had a highly significant effect on IAA production (Supplementary Table 2; Supplementary Fig. 2).

Previous studies have also reported IAA production in AD processes. For instance, Scaglia *et al.* (2015) found 9.94 mg/L in the neutral hydrophobic fraction of pig slurry digestate, which stimulated cucumber root growth. Ertani *et al.* (2013) reported 0.0057 mg/L in digestates from agro-livestock waste, which improved corn root development. Li *et al.* (2016) obtained 23.41 mg/L at 32 days in pig manure digestate.

Although the production of phytoregulators by anaerobic microorganisms is not as well-established as it is by aerobic microorganisms, some advances have been made in the production of IAA. Li *et al.* (2018) investigated IAA metabolism in anaerobic reactors and concluded that IAA is produced from L-tryptophan. They also found that adding an exogenous carbon source inhibits the transformation of IAA to skatole, thereby enhancing IAA production.

Our results suggest that the initial pH has a strong influence on the production of IAA. To improve IAA production, future research could examine the effects of lower initial pH values, longer fermentation times at pH 5.5, or shorter fermentation intervals (0–4 days) at pH 6.5.

3.5 Abscisic acid

ABA was not detected in any of the evaluated treatments in this study. This is in accordance with Castro-Sierra *et al.* (2024), they did not detect ABA using bovine or swine manure without inoculum. This contrasts with the results reported by Li *et al.* (2016) who found that ABA was produced during the anaerobic digestion of dairy, chicken, and cow manure with the addition of sewage sludge as external inoculum source. In the present study, however, no external inoculum source was used thus, the rumen microbiome contained in the dairy manure performed the AD process. Although knowledge regarding the anaerobic production of phytoregulators is limited, it is well documented that the inoculum source strongly affects the profile and production of volatile fatty acids during the AD process (Ding *et al.*, 2022).

Since Li *et al.* (2016) reported ABA production from three evaluated substrates (dairy, chicken, and cow manure), the inoculum source is likely one of the determining factors of ABA production. Thus, evaluating AD using different inoculum sources, different inoculum-to-substrate ratios, and different operational AD conditions could be explored to reach ABA production.

Conclusions

Both initial pH and digestion time were found to highly influence the production and profile of phytoregulators during anaerobic digestion of DM. A maximum GA_3 concentration of 124.86 mg/mL was obtained at initial pH 8.5 and 8 days, while IAA reached 5.52 mg/L at initial pH 5.5 and 20 days. ABA was not detected under the evaluated conditions. The observed acidification and absence of methane in all AD treatments suggest that GA_3 and IAA were produced during the hydrolysis-acidogenic stage of anaerobic digestion.

These findings can be used to conduct controlled AD processes to produce digestates enriched in GA_3 and IAA, which could be applied in agriculture to enhance crop development. Furthermore, the AD of dairy manure for GA_3 production could be an interesting alternative to conventional fermentation due to its lower energy requirements and use of residual substrates. However, further research is needed to optimize process parameters, evaluate substrate pretreatment, and develop efficient downstream processing and purification methods.

Beyond the production of GA_3 and IAA, AD also offers environmental benefits by revalorizing DM, a waste material with potential ecological impact if improperly disposed. Thus, the integration of phytoregulator production into AD systems contributes to the circular economy and sustainable waste management strategies.

Acknowledgements

The authors would like to thank the Secretaría de Investigación y Posgrado from the Instituto Politécnico Nacional and the Secretaría de Ciencias, Humanidades, Tecnología e Innovación, México.

References

Almeida-Streitwieser, D. (2017). Comparison of the anaerobic digestion at the mesophilic and thermophilic temperature regime of organic wastes from the agribusiness. *Bioresource Technology* 241, 985-992. https://doi.org/10.1016/j.biortech.2017.06.006

American Public Health Association (APHA). (2017). *Standard methods for the examination of water and wastewater.* American Public Health Association, Washington DC.

Alvarado-Reyna, P.M., Albalate-Ramírez, A., García-Balandrán, E.E., Escamilla-Alvarado, C., Galván-Arzola, U., Miramontes-Martínez, L.R. and Rivas-García, P. (2024). Evaluation of the reaction capacity of early warning indicators to failures in biogas production systems. *Revista Mexicana de Ingeniería Química* 23(3), 1-19. https://doi.org/10.24275/rmiq/Bio24313

Bibi, A., Ullah, M.S. and Mahmood, A. (2025). Indole-3-acetic acid improves growth, physiology, photosynthesis, and ion balance under cadmium stress in *Sorghum bicolor*. *Scientific Reports* 15, 33971. https://doi.org/10.1038/s41598-025-10900-7

Castro-Ramos, J.J., Solís-Oba, A., Solís-Oba, Calderón-Vázquez, C.L., Higuera-Rubio, J. M. and Castro-Rivera, R. (2022). Effect of the initial pH on the anaerobic digestion process of dairy cattle manure. *AMB Express* 12, 162. https://doi.org/10.1186/s13568-022-01486-8

Castro-Sierra, A., Espinosa-Solares, T., Houbron, E., Castro-Rivera, R., Azcárraga-Salinas, B.Y. Pacheco-Ortíz, J.A. and Solís-Oba, M.M. (2024). Production of phytoregulators during anaerobic digestion of bovine and porcine manures. *Revista Mexicana de Ingeniería Química* 23(3), 1-11. https://doi.org/10.24275/rmiq/Bio24289

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K. and Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. *Journal of Integrative Plant Biology* 62, 25-54. https://doi.org/10.1111/jipb.12899

Chuenchart, W., Timilsina, A. P., Ge, J. and Shah, A. (2024). Advances in biorefinery of cattle manure for value-added products. *Fermentation* 10(11), 568. https://doi.org/10.3390/fermentation10110568

Darwin, C.W. and Cord-Ruwisch, R. (2019). Anaerobic acidification of sugar-containing wastewater for biotechnological production of organic acids and ethanol. *Environmental Technology* 40(25), 3276-3286. https://doi.org/10.1080/09593330.2018.1468489

Desai, S.A. (2017). Isolation and characterization of gibberellic acid (GA_3) producing rhizobacteria from sugarcane roots. *Bioscience Discovery* 8(3), 488-494. https://jbsd.in/Vol%208%20No%203/Sagar488-494.pdf

Deublein, D. and Steinhauser, A. (2008). Formation of biogas. In: *Biogas from waste and renewable resources,* (D. Deubleien and A. Steinhauser, eds.), Pp. 87-137. Wiley-VCH, Germany.

Ding H., Barlaz, M.A., Reyes, F.L. and Call, D.F. (2022). Influence of inoculum type on volatile fatty acid and methane production in short-term anaerobic food waste digestion tests. *ACS Sustainable Chemistry & Engineering* 10(51), 17071–17080. https://doi.org/10.1021/acssuschemeng.2c04080

Dospatliev, L., Aatanasoff, A., Kostadinova, G., Penev, T., Miteva, T. and Kirov, V. (2015). Factors associated with change in pH, ammonia and total nitrogen of manure mass in high performance dairy cows. *Veterinarija ir Zootechnika* 70(92).

Emamverdian, A., Ding, Y. and Mokhberdoran, F. (2020). The role of salicylic acid and gibberellin signaling in plant responses to abiotic stress with an emphasis on heavy metals. *Plant Signaling & Behavior* 15(7), 1777372. https://doi.org/10.1080/15592324.2020.1777372

Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M. and Nardi, S. (2013). Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (Zea mays L.) plantlets. *Journal of Geochemical Exploration* 129, 103-111. https://doi.org/10.1016/j.gexplo.2012.10.001

Feng, K., Li, H. and Zheng, C. (2018). Shifting product spectrum by pH adjustment duringlong-term continuous anaerobic fermentation of food waste. *Bioresource Technology* 270, 180-188. https://doi.org/10.1016/j.biortech.2018.09.035

Font-Palma, C. (2019). Methods for the treatment of cattle manure a review. *C Journal of Carbon Research* 5(2), 27. https://doi.org/10.3390/c5020027

Glover, C.J., McDonnell, A., Rollins, K.S., Hiibel, S.R. and Cornejo, P.K. (2023). Assessing the environmental impact of resource recovery from dairy manure. *Journal of Environmental Management* 330, 117150. https://doi.org/10.1016/J.JENVMAN.2022.117150.

Grand View Research. (2022). *Gibberellins market (2020-2025).* Available at: https://www.grandviewresearch.com/industry-analysis/gibberellins-market. Accessed: March 26, 2026.

Gutierrez, C.K., Matsui, G.Y., Lincoln, D.E. and Lovell, C.R. (2009). Production of the phytohormone indole-3-acetic acid by estuarine species of the genus vibrio. *Applied and Environmental Microbiology* 75(8), 2253-2258. https://doi.org/10.1128/AEM.02072-08

Hernández-Eugenio, G., Espinosa-Solares, T., López-Ortiz, C., Meneses-Reyes, J.C. and Ochoa-Bernal, T.G. (2025). Changes in microbial diversity and methane yield caused by overloading in systems of chicken litter, microalgae oil-free and glycerol in co-digestion. *Revista Mexicana de Ingeniería Quím*ica 24(2), 1-11. https://doi.org/10.24275/rmiq/IA25488

Islam, M.S., Hasan, M.K., Islam, M.R., Chowdhury, M.K., Pramanik, M.H., Iqbal, M.A., Rajendran, K., Iqbal, R., Soufan, W., Kamran, M., Liyun, L. and El-Sabagh, A. (2023). Water relations and yield characteristics of mungbean as influenced by foliar application of gibberellic acid (GA_3). *Frontiers in Ecology and Evolution* 11, 1048768. https://doi.org/10.3389/fevo.2023.1048768

Jin, Q. and Kirk, M.F. (2018). pH as a primary control in environmental microbiology: thermodynamic perspective. *Frontiers in Environmental Science* 6, 1-15. https://doi.org/10.3389/fenvs.2018.00021

Karishma, S., Arya, M.S., Reshma, U.R., Anaswara, S.J. and Thampi, S.S. (2019). Impact of plant growth regulators on fruit production. *International Journal of Current Microbiology and Applied Science* 8(2), 800-814. https://doi.org/10.20546/ijcmas.2019.802.092

Li, X., Guo, J., Pang, C. and Dong, R. (2016). Anaerobic digestion and storage influence availability of plant hormones in livestock slurry. *ACS Sustainable Chemistry & Engineering* 4(3), 719–727. https://doi.org/10.1021/acssuschemeng.5b00586

Li, X., Guo, J., Dong, R. and Zhang, W. (2018). Indolic derivatives metabolism in the anaerobic reactor treating animal manure: pathways and regulation. *ACS Sustainable Chemistry & Engineering* 6(9), 11511-11518. https://doi.org/10.1021/acssuschemeng.8b01601

Liu H, Wang, J., Liu, X., Fu, B., Chen, J. and Yu, H.Q. (2012). Acidogenic fermentation of proteinaceous sewage sludge: Effect of pH. *Water Research* 46(3), 799-807. https://doi.org/10.1016/j.watres.2011.11.047

Liu, C., Guo, T., Chen, Y., Meng, Q., Zhu, C. and Huang, H. (2018). Physicochemical characteristics of stored cattle manure affect methane emissions by inducing divergence of methanogens that have different interactions with bacteria. *Agriculture Ecosystems & Environment* 253, 38-47. https://doi.org/10.1016/j.agee.2017.10.020

Ma, H., Chen, X., Liu, H., Liu, H. and Fu, B. (2016). Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: Alkaline or neutral pH?. *Waste Management* 48, 397-403. https://doi.org/10.1016/j.wasman.2015.11.029

Mahamud, M.A., Imran, S., Paul, N.C., Rabbi, R.H.M., Jahan, N., Sarker, P., Hoque, M.N., Sumi, M.J., Asaduzzaman, M., Rehman, S.U, Brestic M., Bárek, V., Skalicky, M., Hossain, A. and Rhaman, M.S. (2025). An overview and current progress of gibberellic acid-mediated abiotic stress alleviation in plants. *Plant Soil and Environment* 71(7), 453–479. https://doi.org/10.17221/137/2025-PSE

Nguyen, C.T., Dang, L.H., Nguyen, D.T., Tran, K.P., Giang, B.L. and Tran, N. Q. (2019). Effect of GA_3 and Gly plant growth regulators on productivity and sugar content of sugarcane. *Agriculture* 9(7), 136. https://doi.org/10.3390/agriculture9070136

Oliveira, J., Rodrigues, C., Vandenberghe, L.P.S., Camara, M.C., Libardi, N. and Soccol, C.R. (2017). Gibberellic acid production by different fermentation systems using citric pulp as substrate/support. *BioMed Research International* 5191046, 1-8. https://doi.org/10.1155/2017/5191046

Parihar, S.S., Saini, K.P.S., Lakhani, G.P., Jain, A., Roy, B., Ghosh, S. and Aharwal, B. (2019). Livestock waste management: a review. *Journal of Entomology and Zoology Studies* 7(3), 384-393. https://www.entomoljournal.com/archives/2019/vol7issue3/PartG/6-6-95-692.pdf

Pore, S., Shetty, D., Arora, P., Maheshwari, S. and Dhakephalkar, P. (2016). Metagenome changes in the biogas producing community during anaerobic digestion of rice straw. *Bioresource Technology* 213, 50-53. https://doi.org/10.1016/j.biortech.2016.03.045

Raja-Ram, N. and Nikhil, G.N. (2022). A critical review on sustainable biogas production with focus on microbial-substrate interactions: bottlenecks and breakthroughs. *Bioresource Technology Reports* 19, 101170. https://doi.org/10.1016/j.biteb.2022.101170

Rodrigues, C., Vandenberghe, L.P.S., Teodoro, J., Oss, J.F., Pandey, A. and Soccol, C.R. (2009). A new alternative to produce gibberellic acid by solid state fermentation. *Brazilian archives of biology and technology* 52, 181-188. https://doi.org/10.1590/S1516-89132009000700023

Scaglia, B., Pognani, M. and Adani, F. (2015). Evaluation of hormone-like activity of the dissolved organic matter fraction (DOM) of compost and digestate. *Science of The Total Environment* 514, 314-321. https://doi.org/10.1016/j.scitotenv.2015.02.009

Shi, T.Q., Peng, H., Zeng, S.Y., Ji, R.Y., Shi, K., Huang, H. and Ji, X.J. (2017). Microbial production of plant hormones: Opportunities and challenges. *Bioengineered* 8(2), 124-128. https://doi.org/10.1080/21655979.2016.1212138

Siddikee, M.A., Hamayun, M., Han, G.H. and Sa, T. (2010). Optimization of gibberellic acid production by *Methylobacterium oryzae* CBMB20. *Korean Journal of Soil Science and Fertilizer* 43(4), 522–527. https://koreascience.kr/article/JAKO201020842653780.pdf

Solé-Bundó, M., Cucina, M., Folch, M., Tàpias, J., Gigliotti, G., Garfí, M. and Ferrer, I. (2017). Assessing the agricultural reuse of the digestate from microalgae anaerobic digestion and co-digestion with sewage sludge. *Science of The Total Environment* 586, 1-9. https://doi.org/10.1016/j.scitotenv.2017.02.006

Soyer, G. and Yilmaz, E. (2020). Waste management in a dairy cattle farms in Aydın region. Potential of energy application. *Sustainability* 12(4), 1614. https://doi.org/10.3390/su12041614

Srisowmeya, G., Chakravarthy, M. and Nandhini, D.G. (2020). Critical considerations in two-stage anaerobic digestion of food waste a review. *Renewable and Sustainable Energy Reviews* 119, 109587. https://doi.org/10.1016/j.rser.2019.109587

Surra, E., Bernardo, M., Lapa, N., Esteves, I.A.A.C., Fonseca, I. and Mota, J.P.B. (2019). Biomethane production through anaerobic co-digestion with Maize Cob Waste based on a biorefinery concept: a review. *Journal of Environmental Management* 249, 109351. https://doi.org/10.1016/j.jenvman.2019.109351

Wang, H.N., Ke, X., Zhou, J.P., Liu, Z.Q. and Zheng, Y.G. (2022). Recent advances in metabolic regulation and bioengineering of gibberellic acid biosynthesis in *Fusarium fujikuroi*. *World Journal of Microbiology and Biotechnology* 38, 131. https://doi.org/10.1007/s11274-022-03324-2

Wang, J., Liu, L. and Zhang, H. (2024). Exogenous indole-3-acetic acid promotes the plant growth and accumulation of selenium in grapevine under selenium stress. *BMC Plant Biology* 24, 426. https://doi.org/10.1186/s12870-024-05105-5

Wang, L.R., Zhu, S.T., Tang, J.Z.H., Nie, Z.K., Ye, C., Zhang, X.L. and Shi, T.Q. (2025). High-level production of abscisic acid by multi-strategy combination optimization in *Botrytis cinerea*. *Biotechnology and Bioengineering* 122, 3298–3308. https://doi.org/10.1002/bit.70056.

Yu, T., Xin, Y. and Liu, P. (2024). Exogenous abscisic acid (ABA) improves the filling process of maize grains at different ear positions by promoting starch accumulation and regulating hormone levels under high planting density. *BMC Plant Biology* 24, 80. https://doi.org/10.1186/s12870-024-04755-9

Zulfiqar, B., Raza, M.A.S., Saleem, M.F., Ali, B., Aslam, M.U., Al-Ghamdi, A.A., Elshikh, M.S., Hassan, M.U., Toleikienė, M., Ahmed, J., Rizwan, M. and Iqbal, R. (2024).Abscisic acid improves drought resilience, growth, physio-biochemical and quality attributes in wheat (*Triticum aestivum L.*) at critical growth stages. *Scientific Reports* 14, 20411. https://doi.org/10.1038/s41598-024-71404-4