Production of VFAs through anaerobic digestion of cattle and swine manure

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Abstract: Anaerobic digestion (AD) is a key biotechnological process for the valorization of organic residues, as it reduces their pollutant load and generates valuable products such as volatile fatty acids (VFAs). These compounds have industrial applications as precursors for biopolymers, solvents, and biofuels. In this study, the kinetics of VFAs were evaluated using cattle manure (CM) and swine manure (SM), incubated at 37 °C for 18 days. A completely randomized design was applied with ten sampling times and three replicates per treatment. VFA quantification was performed by HPLC, and parameters such as chemical oxygen demand (COD), pH, and nutrient concentrations (N, P, K) were also analyzed. SM reached a maximum production of 1.699 g/L at day 4, whereas CM peaked at 1.817 g/L on day 10. In both substrates, pH exhibited an initial decline, indicating hydrolytic and acidogenic phases, followed by stabilization toward acetogenic and methanogenic stages. Reductions in COD, nitrogen, and phosphorus were also observed, reflecting intense microbial activity. Both manures proved viable, with SM being more efficient at early stages and CM performing better in later phases. These findings provide key information for the design of biorefineries within circular economy frameworks.

Keywords: valorization of waste, acidogenic fermentation, environmental biotechnology

Introduction

Anaerobic digestion (AD) is a widely studied biological process due to its relevance in the sustainable management of organic waste and the production of renewable energy. This process is based on the synergistic activity of microbial consortia that, in the absence of oxygen, transform organic matter into metabolic products such as biogas and valuable intermediate compounds. Its application has expanded globally due to its capacity to reduce the pollutant load of agro-industrial residues, mitigate greenhouse gas emissions, and replace fossil-based energy sources, aligning with the principles of environmental sustainability and the circular economy (Atelge *et al*., 2020).

The AD mechanism consists of four interdependent microbial stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, specialized bacteria degrade complex polymers (such as cellulose, hemicellulose, proteins, and lipids) into soluble monomers. Then, in acidogenesis, these products are fermented into simpler compounds, including volatile fatty acids (VFAs), ethanol, lactate, carbon dioxide (CO_2), and hydrogen gas (H_2). Acetogenesis converts these intermediates into acetate, H_2, and CO_2, and finally, during methanogenesis, methanogenic archaea utilize these compounds to generate methane (CH_4), the main energy component of biogas (Giduthuri & Ahring, 2023; Patel *et al*., 2021).

Within these stages, VFA production is strategically important not only as an intermediate step on the pathway to CH_4 formation but also as an alternative route for obtaining value-added compounds. VFAs—including acetic, formic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and enanthic acids—are low-molecular-weight molecules which can serve as raw materials for the manufacture of biopolymers, solvents, biofuels, food supplements, and chemical additives (Franke-Whittle *et al*., 2014; Harirchi *et al*., 2022). This dual functionality, as both metabolic intermediates and final products, has motivated the development of anaerobic digestion systems specifically oriented toward maximizing their accumulation rather than favoring methanogenesis.

The efficiency and selectivity of the process depend on multiple operational factors such as temperature, pH, digestion time, and critically the type of substrate used. In this context, livestock manures, particularly cattle and swine manure, represent abundant and economically accessible sources of fermentable organic matter. Their nutrient-rich composition, high nitrogen content, and elevated proportion of volatile solids make them ideal candidates for acidogenic fermentation. However, physicochemical differences between these substrates can influence microbial dynamics, the predominant metabolic pathway, and the VFA accumulation rate (Cisneros De La Cueva *et al*., 2021; Wang *et al.,* 2022).

From this perspective, the present study aims to evaluate VFA production during the anaerobic digestion of cattle and swine manure at a constant temperature of 37 °C. To achieve this, different digestion times (0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 days) were established, allowing for a detailed kinetic analysis of the fermentative behavior of each substrate. This evaluation not only enabled comparison of VFA yield, production, and composition between the two manures but also provided key information for optimizing anaerobic systems oriented toward the generation of industrially valuable biocompounds.

Materials and Methods

Cattle manure (CM) and swine manure (SM) samples were collected in Tlaxcala, Mexico. CM had an initial dry matter content of 22.41% and a pH of 8.02, whereas SM exhibited 29.09% dry matter and a pH of 6.15. The samples were subjected to air-drying at ambient temperature (25 ± 5 °C), followed by homogenization by manual grinding in a mortar and subsequent sieving through a MONTINOX No. 20 mesh. Once processed, the samples were stored in sealed glass jars under dark and dry conditions until use.

Experimental design

To evaluate VFA production, a completely randomized design (CRD) was implemented, considering two main factors: substrate type (CM and SM) and digestion time (0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 days), at a constant temperature of 37 °C, with three replicates per treatment, resulting in a total of 60 experimental units. The experiment was conducted in batches using 600-mL glass flasks as reactors, each loaded with 5% substrate (CM or SM), 5% (v/v) inoculum obtained from a previous anaerobic digestion process (Castro-Sierra *et al*., 2024), and distilled water to reach a final working volume of 500 mL. The flasks were purged with N_2 to remove residual oxygen and then hermetically sealed. Incubation was carried out in an ECOSHEL 9165 unit for 18 days, with periodic monitoring every two days.

Physicochemical evaluation of the digestates

The digestates obtained during the anaerobic digestion process were characterized through a comprehensive analysis of their physicochemical properties. The evaluated variables included chemical oxygen demand (COD) and the concentrations of total nitrogen (N), total phosphorus (P), and total potassium (K), which were determined using HACH analysis kits and a HACH DR 2800 spectrophotometer. Moisture content was measured with an OHAUS MB45 thermobalance, while total solids (TS) and volatile solids (VS) were quantified by calcination in a THERMOLYNE 1400 muffle furnace. pH was measured using a HINOTEK PHS-3E potentiometer. All determinations were performed following the standardized protocols in the Standard Methods for the Examination of Water and Wastewater of the APHA (2017).

Determination of total volatile fatty acid (VFAs) production

The digestates were centrifuged at 9000 rpm for 10 minutes at 20 °C using a LABNET PRISM R refrigerated microcentrifuge. Subsequently, 2 mL of the supernatant was collected and filtered through 0.22 µm CORNING syringe filters. The identification and quantification of volatile fatty acids (VFAs) were performed by high-performance liquid chromatography (HPLC) using an Agilent 1100 G1311A system coupled to a REZEX ROA-ORGANIC ACID H^+ (8%) ion-exclusion column. The analysis was conducted under isocratic flow conditions, employing HPLC-grade water acidified with 0.005 M sulfuric acid as the mobile phase, at a flow rate of 0.6 mL/min for a total runtime of 30 minutes. Detection was carried out at 205 nm, with the column maintained at a constant temperature of 65 °C (Colzi & Estrada, 2020; Tabarez *et al*., 2024).

VFAs quantification was based on calibration curves generated from a Sigma-Aldrich reference standard mixture containing the following compounds: acetic acid (99.90% purity), formic acid (98.30%), propionic acid (99.70%), isobutyric acid (99.90%), butyric acid (99.90%), isovaleric acid (100.00%), valeric acid (99.90%), isocaproic acid (99.80%), caproic acid (99.60%), and enanthic acid (99.70%). All standard solutions had certified concentrations of 10.0 ± 0.2 mM.

Results and Discussion

Organic matter and nutrients

Figure 1 shows the kinetics of chemical oxygen demand (A), nitrogen (B), phosphorus (C), and potassium (D) during the anaerobic digestion (AD) of cattle and swine manure at 37 °C. With both substrates, COD showed a progressive decrease (a total reduction of 18.2% in cattle manure and 20.8% in swine manure) throughout the process. Approximately 10% of COD in an anaerobic process is used for biomass synthesis, so its reduction indicates microbial activity during digestion (González & Jurado, 2017). Additionally, this significant decrease reflects effective degradation of easily biodegradable organic matter, which serves as the basis for VFA production (Meegoda *et al*., 2018). Cattle manure maintained higher COD values for a longer period, suggesting a higher content of recalcitrant compounds or slower degradation rate. In contrast, swine manure exhibited a faster COD reduction due to its higher content of readily biodegradable compounds (Lee *et al*., 2023).

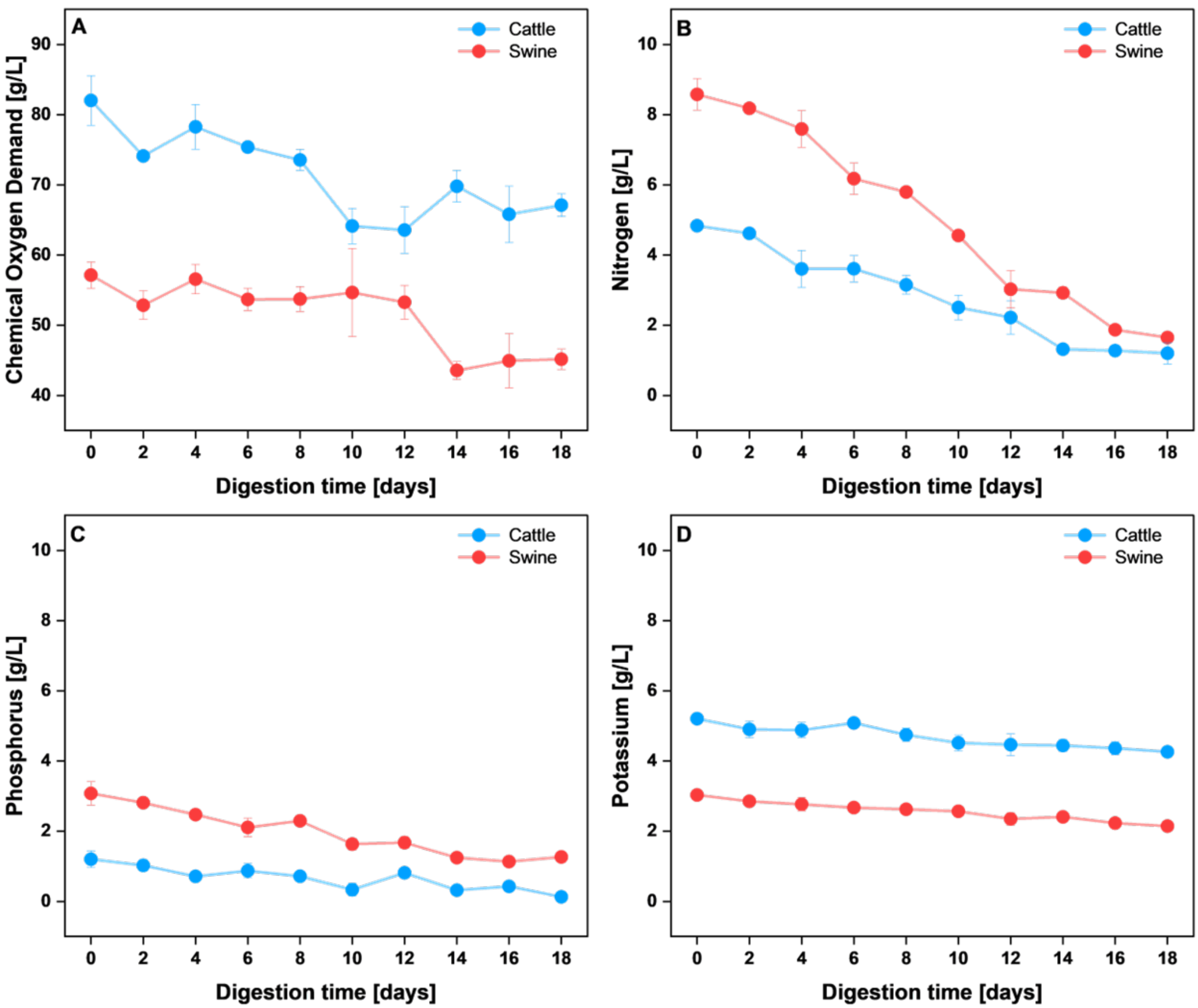

Figure 1. Kinetics of chemical oxygen demand (A), nitrogen (B), phosphorus (C), and potassium (D) during the anaerobic digestion of cattle and swine manure

Similarly, nitrogen exhibited a general decreasing trend (total reduction of 75.0% for cattle manure and 80.2% for swine manure). During AD, nitrogen decreases due to its conversion into gaseous forms such as ammonia (NH_3) and due to microbial assimilation (Kadam *et al*., 2024). The reduction was more pronounced in swine manure, likely attributable to its higher content of soluble proteins, which are more susceptible to ammonification.

Phosphorus also exhibited a continuous decline in both treatments (total change of 91.7% in cattle manure and 58.1% in swine manure), with a greater reduction in cattle manure. This decrease is associated with its incorporation into microbial biomass and the precipitation of insoluble phosphates. Given the magnitude and consistency of this behavior in both substrates, which aligns with recent studies, phosphorus not consumed could potentially be recovered through electrochemical methods (Chen *et al*., 2023).

Unlike the other nutrients, potassium remained relatively stable, with only slight decreases in both manures (17.3% in cattle manure and 30.0% in swine manure). This is because potassium is a mobile element, does not directly participate in microbial transformation reactions, and is mainly present in soluble ionic form. Its slight decrease may be due to incorporation into microbial biomass or minimal losses through leaching. This behavior is consistent with studies reporting the stability of K during anaerobic processes (Tambone *et al*., 2009).

pH and volatile fatty acids

During anaerobic digestion (AD), the pH of both substrates exhibited a pronounced initial decrease (Figure 2, panel A). In the case of swine manure, the initial pH was alkaline (8.96) compared to cattle manure (7.81), but both shifted toward acidic values during the first 4 days, reaching minimums of 6.40 and 5.86, respectively. This corresponds to an intense acidogenic phase, in which the production of volatile fatty acids (VFAs) lowers the pH of the medium (Anukam *et al*., 2019). Subsequently, stabilization was observed in both treatments, with slight fluctuations. The pH of the cattle digestate remained lower throughout the entire period, whereas the swine digestate maintained consistently higher values after day 4, likely indicating lower acid accumulation or better buffering of the organic acids produced. This may be attributed to natural buffering systems such as carbonates and ammonium present in this manure (González-Herrera *et al*., 2021; Solarte *et al*., 2017).

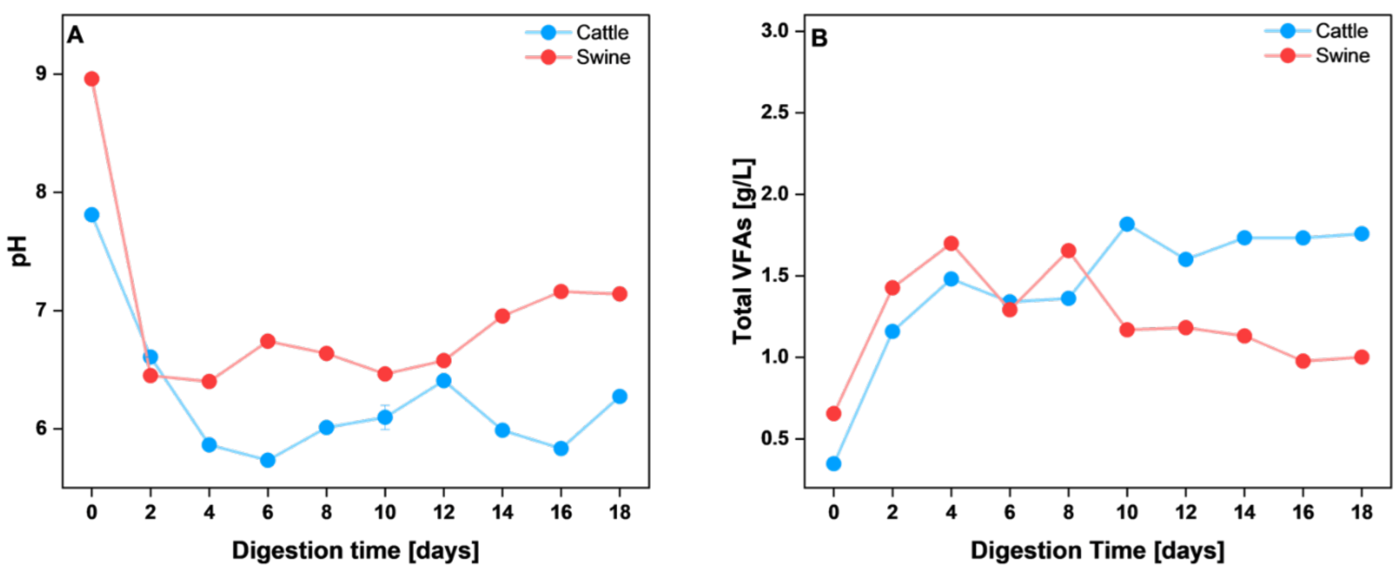

Figure 2. Changes in pH and VFAs production kinetics during the anaerobic digestion of cattle and swine manure

Regarding VFAs production (Figure 2, panel B), both digestates show a sustained increase during the first days of the process. Swine manure exhibits a higher initial VFA production, rising from 0.655 g/L to 1.699 g/L between days 0 and 4, suggesting high availability of fermentable compounds and an active acidogenic microbiota, consistent with its higher content of soluble proteins and lipids (Risberg *et al*., 2017). In contrast, cattle digestate increases more gradually, reaching 1.480 g/L on day 4, but continues rising until reaching its absolute maximum on day 10 with 1.817 g/L. This indicates a slower fermentation kinetics, likely attributable to a matrix with a higher proportion of structural fiber and lower content of easily accessible solutes.

This behavior highlights substantial differences in fermentative dynamics between the two substrates. While swine manure favors early and intense VFA production that stabilizes after day 8, cattle manure maintains sustained and progressive production into later stages, which may be associated with slower hydrolysis of its complex organic components (Bajpai, 2017).

In both cases, the initial decrease in pH correlates with the increase in VFAs, particularly between days 0 and 4. Swine digestate shows a faster pH recovery after the acidic peak, possibly due to a higher buffering capacity or an earlier onset of the methanogenic phase. Conversely, cattle digestate maintains a lower pH over time, which may reflect slower conversion of VFAs to methane or sustained acid accumulation that inhibits methanogenic microorganisms (Castro-Ramos *et al*., 2022).

Conclusions

The results support the use of swine manure (SM) as an ideal substrate for rapid and intensive production of volatile fatty acids (VFAs), whereas cattle manure (CM) may be better suited for prolonged fermentation strategies. SM showed higher efficiency in early VFA accumulation, reaching values up to 1.699 g/L between days 0 and 4, and remaining elevated until day 8. This behavior reflects accelerated acidogenesis, attributable to the greater availability of fermentable substrates. In contrast, CM reached its maximum VFA concentration on day 10 (1.817 g/L), indicating a slower but steady acidogenic kinetics throughout the process. This result suggests that CM may have greater accumulation potential during intermediate stages, likely associated with the gradual release of complex organic compounds.

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