

Article

pH-Regulated Acidogenic Fermentation of Chicken Manure Promotes Lactic Acid and Acetic Acid Production While Limiting Nitrogen Release

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Abstract

Acidogenic fermentation offers a promising approach to convert chicken manure into carbon-rich liquid products for various practical applications. However, the high nitrogen content in chicken manure hinders acidogenesis and limits product utilization due to ammonium accumulation. To address this, a 320-day fermentation experiment was conducted in a two-stage operation (Stage I: uncontrolled pH; Stage II: pH controlled at 5.0). pH adjustment was conducted by adding HCl. The continuously stirred tank fermentation reactor was operated at 37 °C and manually fed daily. Results showed that pH adjustment shifted the metabolic pathway from volatile fatty acid (VFA)-dominated production to lactic acid-enriched production: lactic acid increased from 12.6 to 35.7 g-COD/L, total VFAs decreased by 58%, while acetic acid remained at a relatively high level of chemical oxygen demand (COD) of 17.6 gCOD/L. Total ammonia nitrogen (TAN) decreased from 7.0 to 3.1 g/L, increasing the ratio of soluble COD (SCOD) to TAN from 19:1 to 38:1. The ammonium reduction was primarily due to selective inhibition of uric acid degradation under low pH, while protein hydrolysis remained largely unaffected. These findings demonstrate the potential of pH-regulated chicken manure fermentation liquor as a high-value product.

Keywords: chicken manure; acidogenic fermentation; pH regulation; organic acids; carbon and nitrogen flow

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1. Introduction

The total annual production of livestock and poultry manure in China is approximately 3.8 billion metric tons [1], of which chicken manure alone accounts for more than 110 million metric tons [2]. Compared with traditional anaerobic digestion, acidogenic fermentation can generate high-value products, such as volatile fatty acids and lactic acid, under shorter hydraulic retention times [3,4]. Considering its large quantity and high biodegradability, chicken manure is a promising substrate for acidogenic fermentation for generating high-value bioproducts rather than biogas.

Despite this potential, the intrinsic characteristics of chicken manure impose substantial constraints and limit its use as a substrate for acidogenic fermentation [5]. Chicken

manure contains high levels of nitrogen compounds, primarily proteins and uric acid, which account for approximately 3% of the total solids [6]. The decomposition of this nitrogen matter generates high levels of ammonia nitrogen, which can inhibit acidogenic microbial activity [7]. This also affects the subsequent efficient utilization of chicken manure. COD/TN is a pivotal indicator of the suitability of carbon sources for denitrification. However, the digestate normally has low COD/TAN ratios [8], which is far below the desired value of 6:1 required for use as a carbon source in denitrification [9]. Therefore, the COD/TAN imbalance and nitrogen represent the primary obstacles hindering the utilization of chicken manure acidogenic fermentation product as a viable carbon source. Various methods have been tested in biogas fermentation processes to overcome ammonia toxicity to methanogens. However, the impact of high ammonia levels on the acidogenic fermentation of chicken manure remains rarely studied.

To overcome this drawback, various methods have been developed to control ammonia nitrogen concentrations, such as ammonia stripping, feeding dilution, chemical precipitation, and membrane separation [10]. Compared with these approaches, pH adjustment is more practical due to its low cost and operational feasibility. Existing research consistently indicates that lowering the pH can inhibit the degradation of proteins and uric acid, thereby reducing ammonia nitrogen formation [11,12]. In principle, proteins have complex molecular structures and exhibit low hydrolysis efficiencies under low-pH conditions (<5.0) [13–15]. In traditional biogas fermentation, proteins are largely decomposed into ammonia nitrogen under weakly alkaline pH conditions. In the acidogenic fermentation of chicken manure, uric acid may be the main contributor to ammonia nitrogen formation. However, studies on uric acid degradation under anaerobic conditions remain very limited. A previous study reported that low-pH conditions reduce uric acid degradation efficiency by inhibiting key enzymes such as uricase [11]. It should be noted that most existing studies have utilized model substrates such as bovine serum albumin, which contain simple molecular structures and low levels of impurities [16,17]. Such substrates are insufficient to reflect the actual degradation processes occurring in complex, multi-component substrates like chicken manure. Consequently, conclusions drawn from existing research remain subject to uncertainty.

Although a clear consensus has not yet been reached on how pH regulation affects the targeted production of organic acids, the literature indicates that mildly acidic conditions generally enhance acidogenesis compared with neutral or alkaline environments. Some studies report that pH values around 6.0 promote higher volatile fatty acid (VFA) yields, with butyric acid often becoming the predominant product [18,19]. When the pH falls to 5.5 or below, lactic acid accumulation becomes more pronounced [20,21]. These changes are closely associated with shifts in the dominant microbial community. Hydrolyzing bacteria and acid-producing bacteria are commonly found in anaerobic systems, such as the Bacteroidetes and Firmicutes phyla, and are typically active at pH levels above 6.0 [22,23].

At lower pH levels, lactic acid-producing bacteria, including *Lactobacillus*, *Lactococcus*, and *Streptococcus*, gain dominance and shift metabolic pathways toward lactic acid production [21].

On the other hand, organic acid composition directly influences the efficiency of carbon source utilization, and fermentation broths enriched in both acetic acid and lactic acid are considered particularly suitable for denitrification [24]. Nevertheless, current studies mainly focus on a single class of organic acids (e.g., VFAs or lactic acid), lacking simultaneous quantification of both VFA and lactic acid. More importantly, research on chicken manure acidogenesis is largely limited to short-term batch experiments. To date, there is still a lack of long-term studies systematically examining COD/TN evolution or evaluating

how pH regulation affects the carbon source quality of the effluent. As such, the feasibility of utilizing chicken manure as a viable denitrification carbon source remains unresolved.

Therefore, this study aims to investigate acid production performance by operating a long-term continuous acidogenic fermentation system of chicken manure. Specifically, the research seeks to enhance the accumulation of valuable carbon sources, including acetic acid and lactic acid, by lowering pH, while simultaneously suppressing the degradation of nitrogen-containing compounds to mitigate ammonia nitrogen formation and improve the COD/TN ratio of the fermentation effluent. The proposed strategy involves a pH adjustment to 5.0, regulating the system to generate high concentrations of target acids, thereby capturing the inherent metabolic behavior of complex substrates.

2. Materials and Methods

2.1. Substrate and Inoculum

The chicken manure used in the study was obtained from a chicken farm in Shandong Province of China. Fresh chicken manure was collected and stored in a refrigerator at 4 °C. The raw chicken manure was diluted to 20% total solids and then used as the feed for the reactors. The inoculum was obtained from a long-term, stably operated anaerobic reactor that accepted chicken manure. The total solids and volatile solids of the inoculum were 6.5% and 4.2%, respectively. The inoculum had a pH of 7.8 and a total ammonia nitrogen of 2.1 g/L.

2.2. System Setup and Operation

Serum vials were used as anaerobic reactors with a total volume of 1 L and a working volume of 0.5 L each. The reactors were placed in a thermostatic water bath heater (HX-15, Changliu Beijing, Beijing, China) with the temperature set to 37 °C. The reactors were manually shaken twice per day to ensure homogeneous mixing. The hydraulic retention time (HRT) was maintained at 20 days through daily semi-continuous operation, where 25 mL of the digestate was discharged and replaced with an equal volume of fresh substrate (daily volume = working volume/HRT). The overall experiment lasted for 320 days and was divided into two phases, each with the same feeding strategy and a hydraulic retention time of 20 days. In Stage I (1–66 days), the reactor was operated without pH regulation. During the experiment in Stage II (67–320 days), the reactor pH, initially around 6.0, was adjusted in increments over several days by adding hydrochloric acid (1 mol/L) to bring it down to pH 5.0. No pH regulation was applied thereafter until the fermentation stabilized. The reactor was manually fed, and the effluent was manually withdrawn. During feeding, substrate enters the reactor through a tube with its end submerged below the liquid level to prevent air ingress. In this long-term study, feed and effluent characteristics including pH were routinely analyzed (every 3–5 days) to calculate period-averaged values.

At the beginning of this study, it was speculated that a much lower pH could be established by feeding high-solids chicken manure (20% TS) at a relatively long HRT (20 days), as has been observed for other wastes such as food waste and primary sludge under similar conditions. However, the high nitrogen content might counteract the pH decrease, making the actual pH difficult to predict. Therefore, this study conducted the first stage without pH regulation. Since this stage did not achieve the desired pH, the second stage involved subsequently adding HCl to artificially lower the pH and evaluate the effectiveness of this approach in the same reactor.

2.3. Analytical Methods

Samples were collected daily from the discharged effluent after manual shaking. For soluble components (SCOD, VFAs, and TAN), the samples were centrifuged at 10,000 rpm for 20 min, and the supernatant was filtered through a 0.45 µm membrane before analysis. TS (total solid) and VS (volatile solid) were measured using the oven-drying method (100 °C for 24 h) and the muffle-furnace ignition method (600 °C for 2 h), respectively. The main components of the biogas were determined by means of gas chromatography (Shimadzu GC-8A, Kyoto, Japan). Biogas production was measured using a wet flow meter (LML-1, Jinzhiye, Beijing, China). The effluent from the reactor was measured with a pH meter (Mettler-Toledo, Columbus, OH, USA). Ammonia nitrogen, COD, carbohydrate, and protein contents were analyzed according to the methods used in a previous study [25]. VFA determination was performed using gas chromatography (Shimadzu GC-2100 Plus, Kyoto, Japan). Uric acid was extracted using sodium tetraborate and subsequently determined using a colorimetric method [26]. Samples were extracted with 0.5% borax solution by means of mechanical shaking for 30 min and then filtered. An aliquot of the clear filtrate was mixed with an equal volume of freshly prepared color reagent (containing sodium carbonate, glycine, cupric sulfate, and neocuproine hydrochloride). After exactly 5 min, the absorbance was measured at 450 nm against a reagent blank. Uric acid concentrations were calculated from a standard curve prepared with known amounts of uric acid, which obeyed Beer's law up to 20 µg per assay. The content of lactic acid was determined by means of the colorimetric method using p-hydroxyphenyl after removing interferences using a tungstic acid solution [27].

The biogas production and composition, pH, TAN, VFAs, COD, SCOD, total and soluble carbohydrates, and total and soluble proteins were sampled and measured every 2–3 days. In addition, total nitrogen content, soluble nitrogen content, uric acid, and lactic acid were determined after each stage was stabilized.

In this study, hydrolysis was defined as the solubilization of insoluble polymers. Acidogenesis referred to the generation of VFAs, while acidification included the generation of both VFAs and lactic acid. The efficiencies of hydrolysis, acidogenesis and acidification were calculated based on COD as shown in Equations (1)–(3):

$$\text{Hydrolysis (\%)} = (\text{SCOD}_{\text{out}} + \text{COD}_{\text{CH}_4} - \text{SCOD}_{\text{in}}) / (\text{TCOD}_{\text{in}} - \text{SCOD}_{\text{in}}) \quad (1)$$

$$\text{Acidogenesis (\%)} = (\text{COD}_{\text{VFAout}} + \text{COD}_{\text{CH}_4} - \text{COD}_{\text{VFAin}}) / (\text{TCOD}_{\text{in}} - \text{COD}_{\text{VFAin}}) \quad (2)$$

$$\begin{aligned} \text{Acidification (\%)} \\ = (\text{COD}_{\text{VFAout}} + \text{COD}_{\text{LAout}} + \text{COD}_{\text{CH}_4} - \text{COD}_{\text{VFAin}} - \text{COD}_{\text{LAin}}) / (\text{TCOD}_{\text{in}} - \text{COD}_{\text{VFAin}} - \text{COD}_{\text{LAin}}) \end{aligned} \quad (3)$$

where TCOD_{in} is the total COD of the influent, SCOD_{in} and SCOD_{out} are soluble COD of the influent and effluent, $\text{COD}_{\text{VFAin}}$ and $\text{COD}_{\text{VFAout}}$ refer to VFAs of the influent and effluent calculated via COD, COD_{LAin} and $\text{COD}_{\text{LAout}}$ refer to lactic acid of the influent and effluent calculated via COD, and COD_{CH_4} was calculated based on the principle of 350 mL-CH₄/g-COD under standard conditions. In this study, no methane was produced during the steady phase, with the COD_{CH_4} thus being zero.

The hydrolysis and acidification conversion efficiencies of protein and uric acid were calculated according to Equations (4) and (5) and Equations (6) and (7), respectively [13].

Hydrolysis of protein: Particulate protein → soluble protein → amino acids

Acidification of protein: Amino acids → NH₃

Hydrolysis of uric acid: Particulate uric acid → soluble uric acid

Acidification of uric acid: C₅H₄N₄O₃ = 0.75 CH₃COOH + 3.5 CO₂ + 4 NH₃

$$\text{Protein hydrolysis (\%)} = S_{\text{Proteinout}} + \text{NH}_4^+ - N / 0.16 / T_{\text{Proteinin}} \times 100 \quad (4)$$

$$\text{Protein acidification(\%)} = \text{NH}_4^+ - \text{N}/0.16/\text{T}_{\text{Protein}_{\text{in}}} \times 100 \quad (5)$$

$$\text{Uric acid hydrolysis (\%)} = \text{S}_{\text{Uric acid}_{\text{out}}} + \text{NH}_4^+ - \text{N}/0.33/\text{T}_{\text{Uric acid}_{\text{in}}} \times 100 \quad (6)$$

$$\text{Uric acid acidification (\%)} = \text{NH}_4^+ - \text{N}/0.33/\text{T}_{\text{Uric acid}_{\text{in}}} \times 100 \quad (7)$$

where $\text{T}_{\text{Protein}_{\text{in}}}$ and $\text{T}_{\text{Uric acid}_{\text{in}}}$ are the concentrations of total protein and total uric acid in the influent, $\text{S}_{\text{Protein}_{\text{out}}}$ and $\text{S}_{\text{Uric acid}_{\text{out}}}$ are the concentrations of soluble protein and uric acid in the effluent, $\text{NH}_4^+\text{-N}$ stands for ammonia nitrogen concentration, and 0.16 and 0.33 are the nitrogen contents of protein and uric acid, respectively, calculated on the basis of the ratio of the atomic mass of nitrogen to the total molecular mass of organic matter.

3. Results and Discussion

3.1. VFA and Lactic Acid Production with and Without pH Regulation

The long-term performance of the hydrolysis and acidogenic fermentation is shown in Figure 1. The main results are summarized in Table 1. A short temporary peak in biogas production was observed in Stage I, but not in Stage II. The SCOD was 133.8 g/L when the system was stabilized in Stage I (Figure 1b). At the same time, VFAs accumulated to 48.7 g/L, with acetic acid and butyrate dominating. In addition, a small amount of lactic acid (11.8 g/L) was observed. In Stage II, hydrochloric acid was added to the reactor to lower the pH to 5.0 and no further additions were made. It was expected that the enhanced acidified environment would regulate the acid production and simultaneously suppress the decomposition of proteins. After a gradual decline to 4.3, the pH value continued to rise to about 5.0 from the 138th day to the end of the experiment. At Stage II, the SCOD concentration decreased to 117.6 g/L (Figure 1b). The main component of the total organic acids produced was lactic acid at a concentration of 33.4 g/L (Figure 1c). However, VFAs decreased to 20.6 g/L, and acetic acid dominated entirely (84%) (Figure 1b,d).

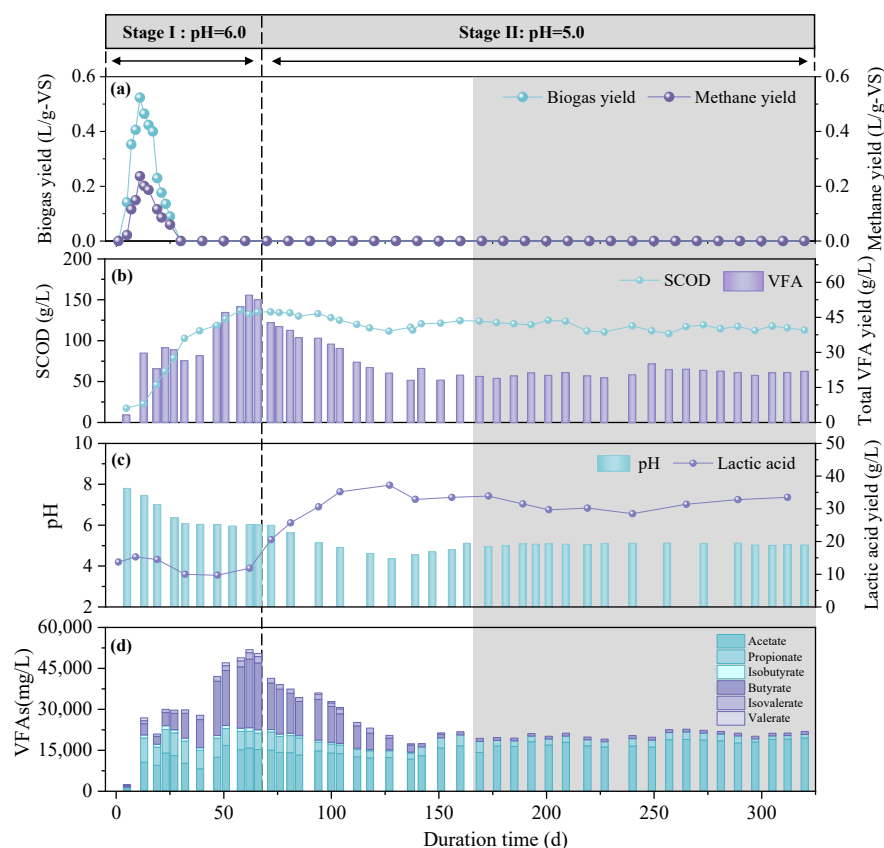
Compared to the VFA profile in Stage I, the most significant difference is that acetic acid became the absolute dominant component. At this pH of 5.0, VFA is produced predominantly as a single dominant component. This shift can be attributed to pH's influence on the expression of enzymes involved in the production of various unsaturated acids during fermentation. It was reported that bacterial cells exhibited increased activity of phosphotransbutyrylase (PTB), the key enzyme controlling butyrate formation, at pH 6.3 [28]. In contrast, at pH 5.0, the activity of phosphotransacetylase (PTA), the main enzyme responsible for acetic acid production, was higher [29].

In addition, another difference is that lactic acid became the primary product in Stage II, accounting for approximately 61% of the total organic acids. This outcome is consistent with established research trends, which show that lactic acid fermentation typically dominates when pH is adjusted to 5.0 or 5.5, with lactic acid accounting for 79% to 85% of the total products [20]. The relatively lower proportion of lactic acid in this study may be attributed to the microbial composition of the inoculum sludge used. Previous studies have suggested that certain inocula are enriched with microbial communities that favor VFA production, potentially enhancing the conversion of substrates into VFAs [20]. The promotion of lactic acid enrichment through pH regulation can be explained by the impact of pH on the reactor microbiota. As reported at pH 5.5–6.0, the fermentation system could enrich *Acidopropionibacterium* and *Prevotella*, which convert substrates into VFAs [20,30]. Lactic acid produced by *Streptococcus* may also be utilized as a substrate to generate VFAs [30]. Conversely, when the pH drops below 5.0, lactic acid-producing bacteria such as *Lactobacillus* become dominant, significantly shifting the fermentation pathway toward lactic acid production [31].

Table 1. Performance in stage I and II.

Parameters	Stage I (1–66 d)	Stage II (67–320 d)
pH	6.0 ± 0.04	5.0 ± 0.15
COD (g/L)	202 ± 7.4	196 ± 7.1
SCOD (g/L)	134 ± 4.1	118 ± 5.3
TAN (g/L)	7.0 ± 0.13	3.1 ± 0.3
TVFA (g/L)	48.7 ± 2.6	20.6 ± 1.3
Acetic acid (g/L)	15.2 ± 1.3	17.3 ± 1.4
Propionic acid (g/L)	6.3 ± 0.4	2.3 ± 0.7
Isobutyric acid (g/L)	1.1 ± 0.1	0
Butyric acid (g/L)	21.9 ± 2.2	1.0 ± 0.1
Isovaleric acid (g/L)	2.0 ± 0.3	0
Valeric acid (g/L)	0.9 ± 0.4	0
Lactic acid (g/L)	11.8 ± 0.2	33.4 ± 0.1

Note: VFA includes acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid. The data were obtained in the stabilized phase of each stage.

**Figure 1.** Biogas yield (a), SCOD-VFA (b), lactic acid (c), and VFA (d) characteristics. The shaded color indicates the stable period.

3.2. COD Material Flow and Carbohydrate Conversion Efficiency

A COD mass balance was established to assess the conversion efficiency of the fermentation system (Figure 2). The mass flow illustrates the VFAs and lactic acid, soluble matter, undegraded matter, and lost matter in both stages. Approximately 65% of the total input COD was converted to SCOD in Stage I, while this value decreased to 57% in Stage II. The SCOD showed a slight reduction of 8% at pH 5.0, aligning with findings from other

studies [32]. Nevertheless, both Stage I and Stage II demonstrated relatively high SCOD, indicating that changes in pH had minimal impact on the hydrolysis process. This result suggests that chicken manure could still maintain a high capacity for soluble organic carbon release under acidic regulation conditions, providing a potential carbon source basis for subsequent denitrification processes.

In Stage I, VFAs and lactic acid accounted for over 60% of the SCOD, whereas in Stage II, this proportion declined to 50%. Although pH regulation reduced the proportion of VFAs and lactic acid in SCOD, this change should not simply be interpreted as a decline in fermentation performance. For the application of fermentation broth as an external carbon source, the composition and quality of available carbon sources are more important than merely increasing the total production of organic acids. Acetic acid and lactic acid produced under acidic regulation conditions are both readily biodegradable carbon sources, and their selective accumulation can improve the suitability of fermentation broth as a carbon source for denitrification.

Further analysis indicates that this change was not attributable to limitations in the hydrolysis process. Specifically, when the pH was reduced from 6.0 to 5.0, the concentration of soluble carbohydrates increased markedly from 3.1 g/L to 6.8 g/L, reflecting substantial accumulation of monosaccharides and oligosaccharides within the reactor. These results demonstrate that lowering the pH diminished the efficiency with which hydrolysis products of carbohydrates were further converted into acidification products. In both stages, inert organic matter in the solid phase accounted for approximately 33% and 38% by weight, respectively, with carbohydrates comprising more than half of this. The particulate carbohydrate remained nearly constant at 42 g/L and 40 g/L across the two stages, implying that carbohydrates were not fully degraded. This phenomenon could be related to the compositional characteristics of chicken manure, in which carbohydrates exist in structurally complex forms such as cellulose and lignin. These complex structures make them resistant to hydrolysis and difficult to utilize within a short retention time [33].

Overall, the COD mass balance results demonstrate that pH regulation altered the carbon distribution pattern during chicken manure acidogenic fermentation. Compared with promoting the generation of large amounts of non-selective mixed fermentation products, the acidic regulation strategy promoted the redistribution of carbon resources toward highly valuable and utilizable carbon source components, such as acetic acid and lactic acid, while maintaining a relatively high level of soluble COD. This carbon flow regulation strategy is of great significance for promoting its application.

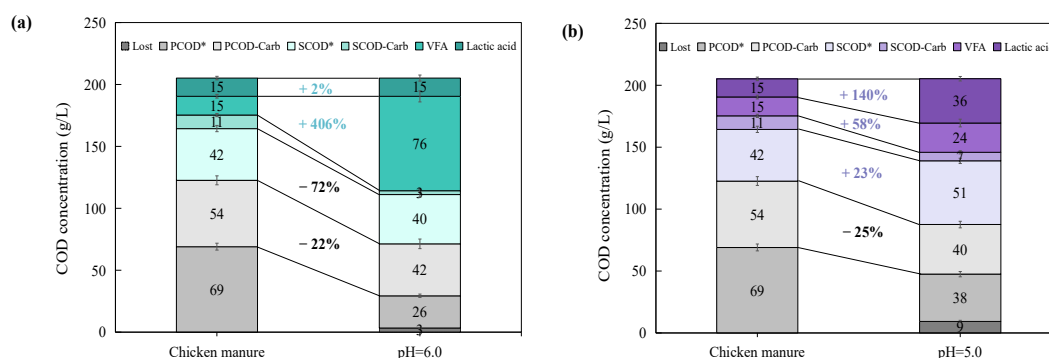


Figure 2. COD mass balance at pH = 6.0 (a) and pH = 5.0 (b). Abbreviations: S, soluble; P, particulate; Carb, carbohydrate; LA, lactic acid. PCOD* represents the fraction of total PCOD excluding PCOD-Carb. SCOD* represents the fraction of total SCOD excluding SCOD-Carb, VFA and lactic acid.

Figure 3a illustrates the effects of pH on carbohydrate hydrolysis and acidification, providing further insight into the phenomena observed during the aforementioned COD conversion process. The hydrolysis efficiencies (Equation (1)) at pH 6.0 and pH 5.0 were 35% and 38%, respectively, suggesting that lowering the pH had a negligible impact on the activity of carbohydrate-hydrolyzing bacteria. Therefore, changes within this pH range (6.0–5.0) may not significantly hinder carbohydrate hydrolysis. However, the acidogenesis (Equation (2)) efficiency of carbohydrates decreased by 35% when the pH was lowered to 5.0 (Figure 3a). It has been reported that *Clostridium sensu stricto* 1 and *Olsenella*, which hydrolyze carbohydrates, are the dominant species at pH 4.0–6.0, whereas *Acetomicrobium*, which can further utilize monosaccharides such as glucose, exhibits high activity at a pH around 7.7 [34,35]. Whether these microorganisms played a key function has not been verified in this study.

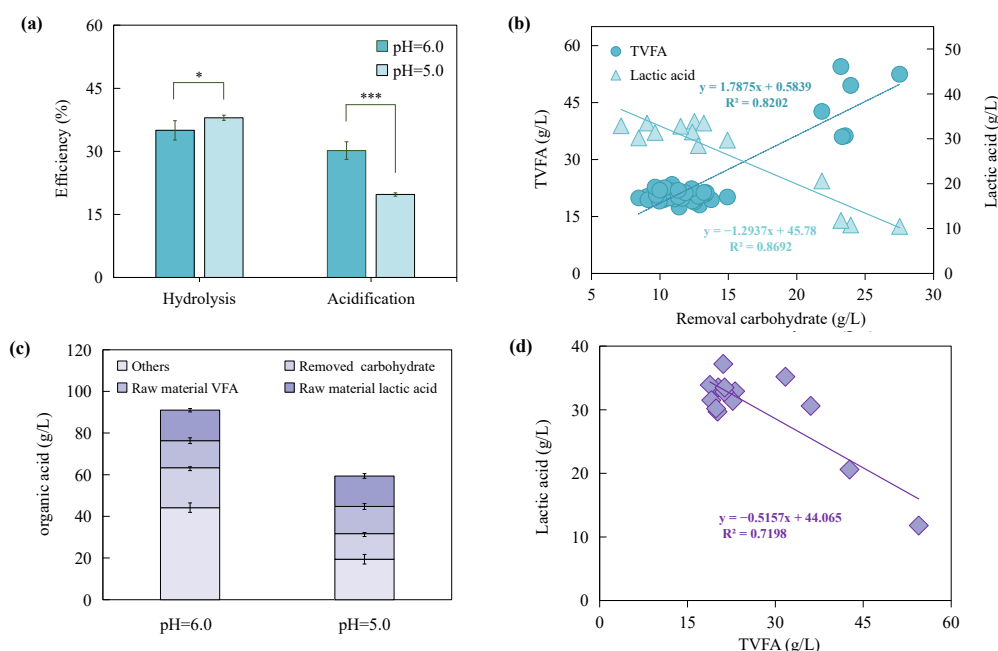


Figure 3. Degradation efficiency of carbohydrates (a,b) and correlation with organic acids (c,d). Note: In Figure 3a, a total of 12 samples were analyzed for the pH 6.0 period, and a total of 23 samples were analyzed for the pH 5.0 period. * indicates significant difference at ($p < 0.05$), *** indicates extremely significant difference at ($p < 0.001$).

Figure 3b illustrates the relationship between VFAs, lactic acid, and removed carbohydrates. This analysis aimed to clarify whether carbohydrate degradation served as the primary carbon source for organic acid production and how pH regulation affected the subsequent carbon conversion pathways. A significant positive correlation was observed between removed carbohydrates and VFAs ($R^2 = 0.820$), indicating that VFAs were mainly derived from carbohydrate degradation. In contrast, a negative correlation was observed between removed carbohydrates and lactic acid. These results demonstrate that carbohydrate degradation contributed substantially to organic acid formation, while the carbon flow derived from carbohydrates was significantly influenced by pH conditions. The contributions of removed carbohydrates to organic acid production were 29% and 38%, respectively (Figure 3c). In addition, a significant negative correlation was observed between VFAs and lactic acid ($R^2 = 0.719$). The negative correlation between VFAs and lactic acid indicates a competitive shift between different fermentation pathways rather than simultaneous enhancement of both products. These results indicate that carbohydrates

were predominantly converted into VFAs under higher pH conditions (6.0), whereas lactic acid became the major product under lower pH conditions (5.0). Therefore, pH regulation functioned as a metabolic selector by modifying the fate of carbohydrate-derived carbon and regulating the formation of different fermentation products, with higher pH promoting VFA-oriented fermentation and lower pH favoring lactic acid accumulation.

3.3. Decomposition and Transformation of Nitrogenous Compounds

The nitrogen flow is illustrated in Figure 4. Proteins account for approximately 46% of the total nitrogen in chicken manure. In the two stages, hydrolysis efficiencies of the protein (Equation (4)) were 36% and 24%, respectively. The particulate protein remained stable at approximately 2.0 g/L across both stages, indicating that the pH did not further enhance protein solubilization. However, the soluble protein concentration rose from 2.1 g/L to 3.1 g/L as the pH decreased from 6.0 to 5.0. These findings suggest that low-pH conditions may hinder the decomposition of soluble proteins, thereby reducing ammonia nitrogen production. Therefore, the decreased protein degradation observed in this study does not indicate that the entire protein conversion process was completely inhibited, but rather suggests that more nitrogen was retained in the system in the form of soluble organic nitrogen. reduced degradation A pH decrease from 6.0 to 5.0 significantly reduced the uric acid degradation efficiency from 68% to 11%, corresponding to a 3.5 g/L decrease in ammonia nitrogen. This is the key reason for the decrease in ammonia nitrogen concentration from 7.0 g/L to 3.1 g/L observed in this study. The reduced degradation efficiency observed at lower pH may be attributed to both decreased uric acid solubility and inhibited microbial activity under acidic conditions, although the present study does not provide sufficient data to distinguish between these two effects. These findings suggest that uric acid may serve as an important direct precursor and key regulatory control for ammonia nitrogen formation during chicken manure fermentation. Previous studies focused on ammonia nitrogen formation associated with protein degradation, whereas the transformation behavior of uric acid in anaerobic chicken manure fermentation received limited attention. The results of this study therefore demonstrate that uric acid metabolism plays a critical role.

In both stages, the concentration of precipitated uric acid was 1.3 g/L and 4.9 g/L, and the concentration of dissolved uric acid was 0.5 g/L and 0.4 g/L, respectively. This represents the possibility that lowering the pH may inhibit the dissolution of uric acid. This phenomenon can be attributed to the nature of uric acid. It was reported that uric acid exists mainly in the form of urate crystals at pH values below 5.75 [36]. Therefore, a decrease in pH to 5.0 results in much less solubilization of uric acid. Ultimately, approximately 64% of uric acid remained in the solid phase, reducing the contact opportunity between uric acid and microorganisms and limiting its subsequent biological degradation.

Additionally, the extent of uric acid degradation is influenced by the activity of microorganisms involved in its catabolism, such as *Clostridium* spp., *Streptococcus przewalskii*, and *Peptostreptococcus* spp., which thrive in an optimal pH range of 6.5–7.8 [37–39]. When the digester becomes more acidic, microbial activity diminishes, reducing uric acid degradation efficiency. Therefore, low pH level inhibited uric acid transformation through a dual mechanism: first, it promoted uric acid precipitation and reduced the bioavailability of dissolved uric acid; second, an acidic environment might inhibit the activity of uric acid-degrading microorganisms, thereby reducing the conversion of uric acid into ammonia nitrogen.

Overall, this study revealed the dual mechanisms by which pH regulation reduced ammonia nitrogen generation during chicken manure fermentation, namely, by inhibiting the degradation of both proteins and uric acid. In conclusion, this study elucidated the mechanism of pH-regulated nitrogen-containing compound retention and degradation

inhibition, providing a new theoretical basis for reducing ammonia nitrogen formation during chicken manure fermentation.

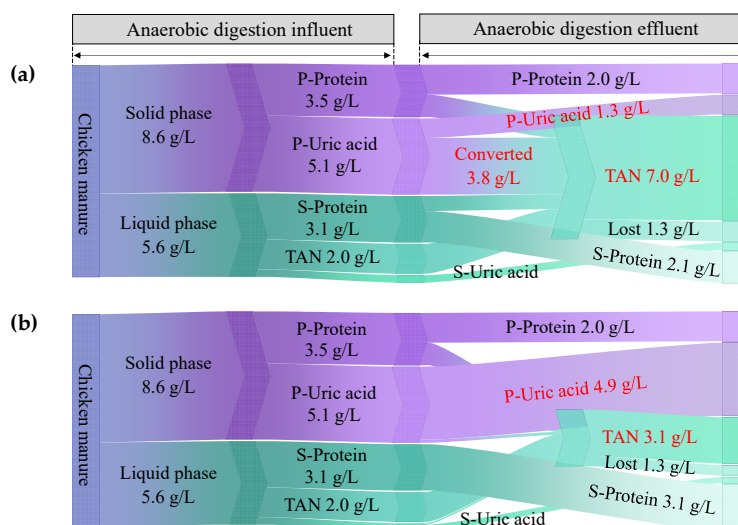


Figure 4. Transformation of nitrogen at pH = 6 (a) and pH = 5 (b).

The effects of different pH values on the hydrolysis efficiency and acidification efficiency of proteins and uric acid are shown in Figure 5a,b. The hydrolysis efficiency of proteins was similar at different pH values, but the acidification efficiency was significantly reduced by 33%. Whether the lower conversion efficiency in acidic environments is caused by the inhibition of protein hydrolysis or the suppression of subsequent acidogenesis is still uncertain. It was reported that the total content of 19 amino acids as protein hydrolysis products decreased from 0.60 g-COD/L to 0.07 g-COD/L when the pH decreased from 7.0 to 5.0, and these amino acids only accumulated transiently in the system before being rapidly fermented into organic acids [16]. Under acidic conditions, the conversion of proteins into amino acids may become a key rate-limiting step for acid production. This indirectly suppresses the release of ammonia nitrogen by limiting the availability of precursors for deamination. The inhibition is related to changes in protein conformational stability and protease (protein hydrolase) activity [40]. As the pH decreased, the increased proton concentration in the solution promoted stronger hydrogen bonding interactions among protein molecules, resulting in a more stable protein conformation and reducing the susceptibility of proteins to enzymatic hydrolysis [17]. In addition, the optimal pH of proteases is generally around 7, and their activity decreases by approximately 65% under pH 5 conditions, further demonstrating from an enzymatic perspective that acidic environments are unfavorable for protein hydrolysis [41].

As for uric acid, both its hydrolysis and acidification efficiencies were significantly decreased by more than 85% after lowering the pH. This demonstrates that both the hydrolysis and acidification of uric acid are inhibited in a strongly acidified environment. Considering that uric acid contributes only a limited proportion to the organic acid carbon source (accounting for only 0.2–3%), but its degradation can release approximately 40% of the total nitrogen in the system, precise regulation of uric acid transformation is of great significance for improving the carbon–nitrogen balance of the chicken manure fermentation system. In summary, this study demonstrated that under precise pH regulation, protein hydrolysis was only slightly affected, whereas uric acid degradation was significantly inhibited. These results indicate that moderate acidification can preferentially restrict uric

acid, an important nitrogen release pathway, while simultaneously maintaining the organic carbon release capacity of soluble proteins to some extent.

Figure 5 shows the relationship between protein and uric acid removal and TAN in the two stages. The coefficient of determination R^2 for both with TAN at different pH values was greater than 0.915, indicating a strong positive correlation between degraded protein, degraded uric acid and TAN. Although certain fluctuations were observed within some data ranges, these variations mainly resulted from the combined contributions of proteins and uric acid to ammonia nitrogen generation, as well as the differences in the degradation processes of these two nitrogen-containing components. However, the overall correlation ($R^2 > 0.915$) confirmed a strong association between protein and uric acid degradation processes and ammonia nitrogen release throughout the entire experimental range. This result further verifies that proteins and uric acid are important sources of TAN formation during chicken manure fermentation and supports the mechanism that pH regulation controls ammonia nitrogen generation by altering the degradation rates of nitrogen-containing organic compounds. In Figure 5c,d, the high R^2 values likely arise from the fact that the data points are concentrated at two distinct pH levels (5.0 and 6.0), which represent the two ends of the regression line. Under such conditions, a high R^2 for linear regression is mathematically expected.

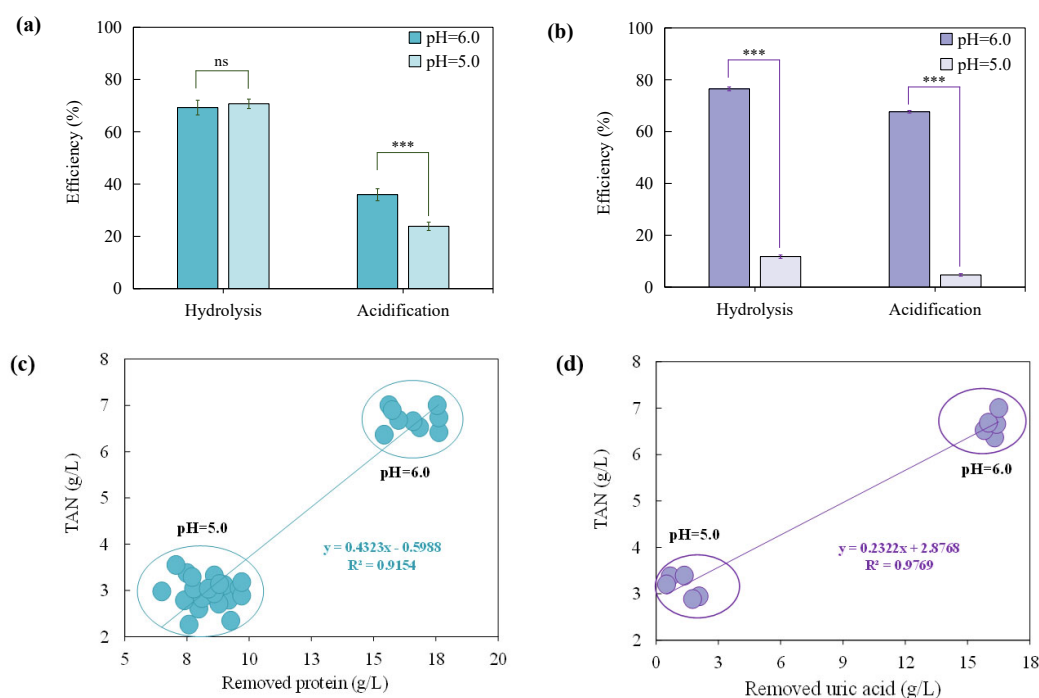


Figure 5. Degradation efficiency of protein (a) and uric acid (b) and correlation with TAN [protein (c) and uric acid (d)]. *** indicates extremely significant difference at ($p < 0.001$), ns means not significant.

3.4. The Variation of the C/N Ratio

The COD and nitrogen concentration are shown in Figure 6. The objective of pH regulation during chicken manure fermentation is to enhance the value of fermentation products by coordinately regulating carbon and nitrogen transformation processes. According to the aforementioned nitrogen migration results, approximately 86% of uric acid and 30% of proteins were retained in the solid phase under low-pH conditions, indicating that an acidic environment effectively limits the further conversion of nitrogen-containing

organic compounds into soluble nitrogen and ammonium nitrogen. Therefore, after solid–liquid separation of the fermentation broth, the liquid fraction enriched with bioavailable organic carbon can be recovered and used as a carbon source for denitrification, while the nitrogen-rich solid digestate has the potential for further development as an organic fertilizer resource. This strategy does not regard nitrogen as waste to be removed; instead, it achieves the coordinated recovery of carbon and nitrogen resources by regulating nitrogen migration pathways, thereby improving the overall resource utilization efficiency of chicken manure.

To further evaluate the suitability of the obtained liquid fraction as a denitrification carbon source, the balance between bioavailable COD and nitrogen concentration should be considered. Therefore, the SCOD/N ratio was used to assess the potential of chicken manure anaerobic fermentation broth as a carbon source for denitrification. In this study, the SCOD/TAN ratio increased from 19:1 under uncontrolled pH conditions (approximately pH 6.0) to 38:1 under pH-regulated conditions (approximately pH 5.0). Combined with the aforementioned COD conversion results, the decrease in pH did not significantly affect SCOD concentration. Therefore, the increase in this ratio was mainly attributed to the reduction in TAN concentration rather than enhanced organic matter hydrolysis, indicating that low pH improved the carbon source quality of the fermentation broth mainly through selective inhibition of ammonium nitrogen formation.

Furthermore, this study revealed different response patterns of TAN and STN under pH regulation. Here, STN refers to the soluble total nitrogen content. Under low-pH conditions, the SCOD/STN ratio increased from 14:1 in the control group to 18:1, representing an approximately 28% improvement. Compared with the substantial increase in the SCOD/TAN ratio, the relatively limited increase in SCOD/STN was mainly due to the accumulation of some soluble proteins in the liquid phase caused by inhibited protein degradation under acidic conditions. Although TAN concentration decreased significantly, STN decreased by only approximately 42%, indicating that pH regulation did not completely remove soluble nitrogen but rather altered the distribution of nitrogen among different forms. The different responses of SCOD/TAN and SCOD/STN to pH regulation further demonstrate that inorganic nitrogen and organic nitrogen undergo different transformation pathways and confirm that pH can selectively regulate nitrogen release during chicken manure fermentation.

Compared with conventional nitrogen control methods, such as ammonia stripping, dilution, chemical precipitation, and membrane separation, pH regulation provides a resource-oriented strategy based on internal regulation during the fermentation process [10]. Conventional methods generally require additional nitrogen removal after ammonium nitrogen formation, which not only increases equipment investment and operational energy consumption but may also result in carbon resource loss. In contrast, pH regulation modifies the transformation pathways of organic nitrogen during the nitrogen release stage, reducing ammonium nitrogen generation at the source while retaining bioavailable organic carbon, thereby achieving simultaneous improvement of carbon source quality and preservation of nitrogen resources. Therefore, pH regulation represents a low-cost, process-intensification, and resource-oriented strategy with promising potential for promoting the high-value utilization of chicken manure.

Figure 7 shows how pH affects the SCOD/TAN and SCOD/STN ratios by influencing the hydrolysis and acidification of carbohydrates, proteins and uric acids. Carbohydrates and proteins are broken down into small organic molecules by various extracellular hydrolases [42]. As mentioned above, the bottleneck limiting the practical application of chicken manure-derived fermentation liquor as a carbon source is not insufficient carbon hydrolysis, but rather the imbalance between carbon and nitrogen. This study confirmed that within the moderately acidic range of pH 5.0–6.0, the hydrolysis processes of the two

major organic substrates in chicken manure were minimally affected by pH, allowing the system to maintain stable soluble COD production and providing a reliable carbon matrix for the formation of high-quality carbon sources.

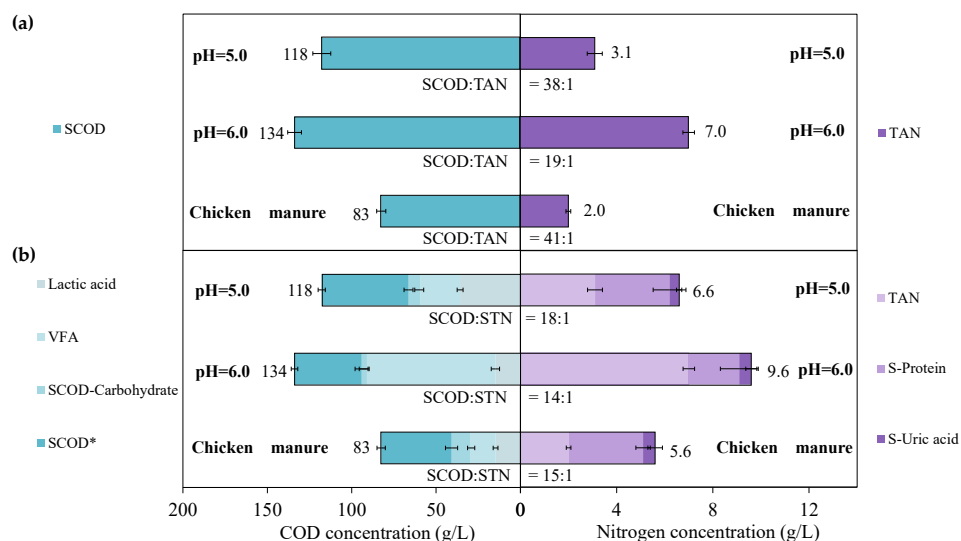
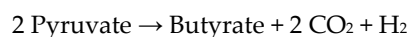
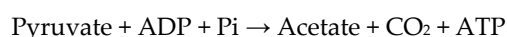
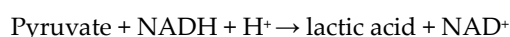


Figure 6. SCOD/TAN (a) and SCOD/STN (b) ratio at pH 6.0 and pH 5.0. Note: The data were calculated based on the average value of each stage. SCOD* represents the fraction of total SCOD excluding SCOD-Carb, VFA, and lactic acid.

During the subsequent acidification of carbohydrates and proteins, pyruvate serves as an important common intermediate metabolite, connecting the degradation of macromolecules such as carbohydrates and amino acids with subsequent short-chain organic acid synthesis reactions [43]. Pyruvate is further used in various pathways to produce VFAs and can also undergo a carbonyl reduction reaction in the presence of lactic acid dehydrogenase (LDH) to produce lactic acid [44]. The metabolic pathways from pyruvate to organic acids are as follows:



In addition, pH shifts activate different metabolic pathways for pyruvate. At pH 6.0, pyruvate primarily yields VFAs, whereas at pH 5.0, the activity of lactic acid bacteria is favored, shifting the main product toward lactic acid. Although similar changes in product distribution have been reported [45], the present study further demonstrates that moderate acidification did not simply result in a complete shift from VFA production to lactic acid production. Instead, high levels of VFAs (20.6 g/L) were still maintained even when lactic acid became the dominant product. This diversified organic acid composition is highly valuable because VFAs and lactic acid exhibit different microbial utilization characteristics during denitrification. Previous denitrification studies have shown that mixed carbon sources containing multiple organic acids possess higher microbial adaptability and stronger resistance to fluctuating loading conditions compared with single carbon sources [46]. Therefore, the pH-regulated fermentation liquor obtained in this study exhibits greater carbon source diversity and enhanced potential for practical application.

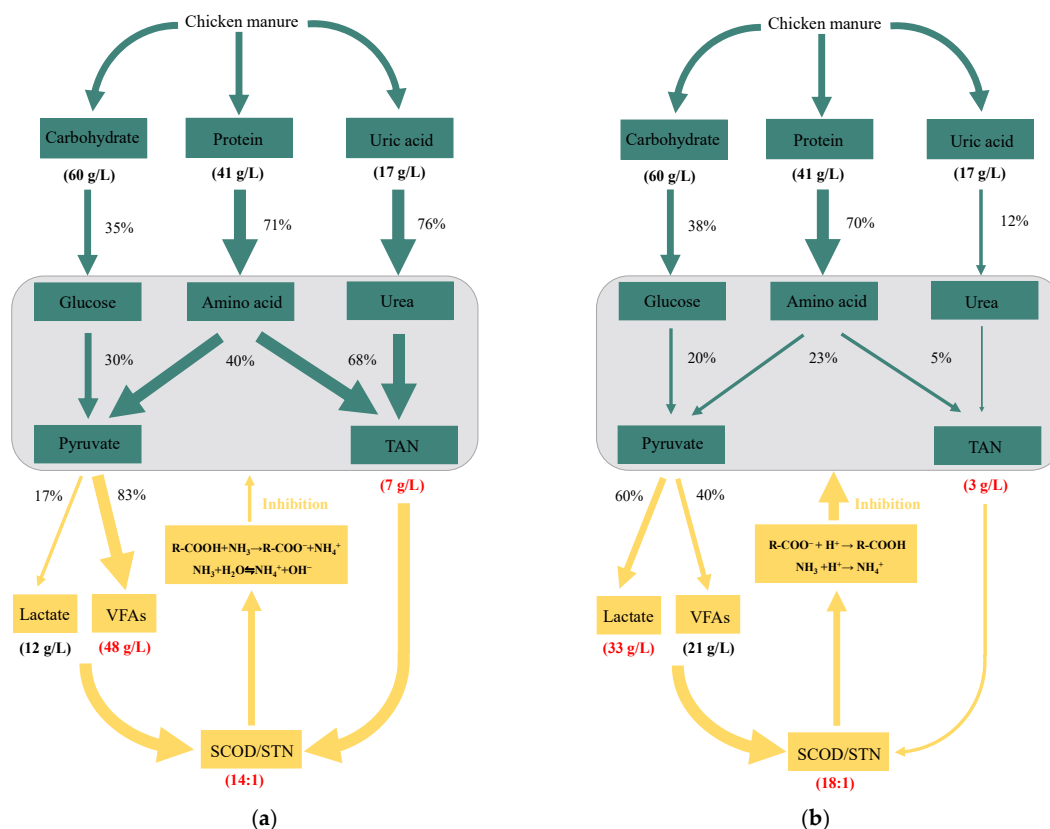


Figure 7. Mechanisms of metabolism for various substances at pH 6.0 (a) and pH 5.0 (b).

The results obtained in this study elucidated the integrated mechanism by which pH regulation enhances the value of chicken manure fermentation products through the synergistic promotion of carbon resource retention and restriction of nitrogen release. This strategy promoted the formation of diverse organic acids and reduced excessive nitrogen release, thereby improving the overall efficiency of nutrient resource recovery.

4. Conclusions

The co-production of VFAs and lactic acid from chicken manure through acidogenic fermentation is often hindered by the decomposition of nitrogenous materials and the resulting higher pH conditions. This study found that the production of these two target acids could be significantly enhanced by regulating the pH to create a suitable acidic environment. Simultaneously, the degradation of nitrogenous materials was suppressed under lower pH, leading to an increased SCOD/N ratio. This strategy promotes the acidogenic fermentation of chicken manure, yielding high-value products suitable for versatile applications, such as serving as a carbon source for wastewater denitrification.

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Institutional Review Board Statement: This paper examines the anaerobic conversion of animal manure to volatile fatty acids and lactic acid. Since the study involves neither human nor animal experiments, no ethical approval is required.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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