

## CHANGE OF COD AND VFAS CONCENTRATION DURING THE SEQUENTIAL BATCH HIGH-TEMPERATURE ANAEROBIC DIGESTION OF CHICKEN MANURE AND STRAW

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**Abstract.** A series of high-temperature combined anaerobic digestion tests with different mass ratios were carried out using chicken manure and straw with a high C/N ratio as the raw materials. The changing trends of CODs, CODr, CODs/CODr and VFAs and the concentration of each component were analyzed during the high-temperature anaerobic digestion of chicken manure and straw using a sequencing batch reactor. The results showed that the COD<sub>T</sub> of the fermentation broth decreased significantly in the first 15 days, and the values of COD<sub>T</sub> decreased from 3584.45, 3132.28, 3355.45 and 2987.39 mg/L in the initial stage to 1756.28, 1532.45, 1607.28 and 1528.33 mg/L, respectively. The degradation rates of COD<sub>T15</sub> were 51.06, 55.09, 52.11 and 48.84%, respectively. At the end of the reaction (50 d), the values of the four groups of COD<sub>T</sub> were 1223.10, 903.21, 1095.39 and 1333.46 mg/L, respectively. As the reaction proceeded to the seventh day, the maximum concentration, the VFAs of the digestive fluids from R1, R2, R3, and R7 were 3032.39, 3346.75, 3245.12 and 2794.03 mg/L, respectively. As the reaction completed, (50 d), the VFAs of the digestive fluids were 1558.34, 1547.37 and 1335.58 mg/L, respectively, in which the contents of formic acid, acetic acid, propionic acid, lactic acid, and butyric acid were 9.19-9.97%, 20.58-22.13%, 15.74-18.44%, 22.86-25.50% and 25.56-30.02%, respectively.

**Keywords:** chicken manure, co-anaerobic digestion, biodegradability, gas properties

### Introduction

In recent years, the development of animal husbandry in China has led to a total annual production of livestock manure exceeding 2 billion tons (Chu et al., 2010). The inappropriate treatment of these manures will negatively affect the living environment (Li et al., 2016). Crop straw and livestock manure are the two most important types of biomass resources in China. For the anaerobic digestion of chicken manure and straw, existing studies mainly focuses on the treatment of raw materials (Linke, 2006; Naranjo et al., 2011; den Boer et al., 2012), improvement of reactor and medium-temperature anaerobic fermentation (Duan et al., 2016), and regulation of nutrients (Tauseef et al., 2013; Nasir et al., 2012; Jiménez et al., 2003). Nutrient regulation can be controlled by adding chemical reagents such as urea and ammonium bicarbonate, as well as mixing various raw materials. Based on the physical and biochemical characteristics of different raw materials, such as water content, carbon to nitrogen ratio and refractory or perishability, it can properly optimize the fluidization characteristics and nutrient structure of the fermentation material, and avoid acid suppression of perishable

materials by using the appropriate proportion. The previous studies focused on the gas production characteristics of rice straw and chicken manure with different mass ratios under medium-temperature conditions. However, the parameter changes in a combined anaerobic digestion process under high-temperature conditions were not analyzed.

In this study, a self-designed bio-reactor for the anaerobic methanogenic reaction was used. The high-temperature combined anaerobic digestion tests with different mass ratios were carried out using livestock manure, i.e., chicken manure and straw with high C/N as the raw materials. The changing trend of soluble chemical oxygen demand (CODs), total chemical oxygen demand (COD<sub>T</sub>), CODs/COD<sub>T</sub> and volatile fatty acids (VFAs) and the concentration of each component were analyzed during high-temperature anaerobic digestion of chicken manure and straw using a sequencing batch reactor. This work will provide the basis for the mixing of raw materials for combined high-temperature dry fermentation process and further engineering application.

## Materials and method

### *Experimental materials and inoculum*

Chicken manure in the experiment came from a chicken farm in Beipiao County, Chaoyang City, Liaoning Province, China. After careful screening to remove impurities, such as stones, chicken manure collected for 3 days in a row was sent to the laboratory directly and kept at 4 °C in refrigerator. After intensive mixing in the laboratory, the collected chicken manure was used as the experimental material for high temperature anaerobic digestion. The extra 500 kg of the material was packaged as 1 kg bags and frozen in refrigerator for a further experiment of continuous high temperature dry fermentation of chicken manure (Chen et al., 2016). Stalks used in the experiments, which were from a farm in Shenbei new district, Shenyang City, Liaoning Province, were shipped to the laboratory and cut and ground to 80 mesh.

The inoculated microorganisms in the experiments were from anaerobic digested mud in a northern waste water treatment factory in Shenyang City, Liaoning Province, China. The inoculated active mud was transferred to an airtight plastic container and its temperature decreased to room temperature of about 20 °C or so during the transportation process, whereas the inoculated mud still maintained active. In the laboratory, the inoculated mud was cultivated and acclimatized at 55 °C. The acquired 5 L of active mud was transferred to a 25 L air-tight plastic bag for acclimatization. After 3 d cultivation at constant 55 °C, 2.5 kg of fresh chicken manure, which was taken in advance and kept at room temperature, was added into active mud after acclimatization for 10 d cultivation. In addition, 5 kg of fresh chicken manure kept at room temperature was acclimatized and cultivated for 10 d for a further use. Dry material masses of chicken manure, stalks, and inoculated mud were determined after heating in air dry oven for 24 h at constant temperature of 105 °C (Duan et al., 2018). The organic content was determined after 4 h heating in the muffle furnace at constant 550 °C. Main parameters of the wet basic state of chicken manure, stalks, and active mud are shown in *Table 1*.

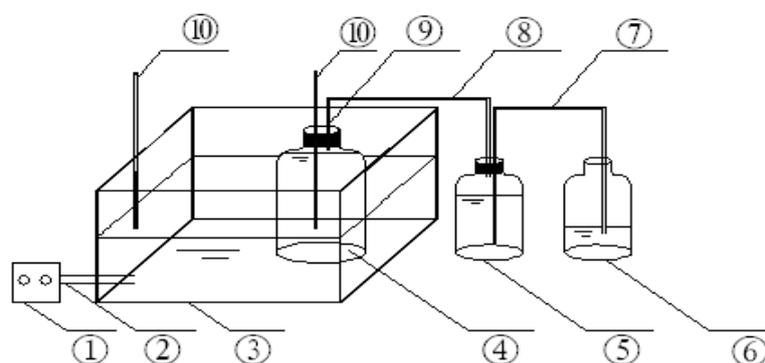
### *Experimental equipment*

A small scale biological and chemical reactor for anaerobic fermentation, which was designed by us, was used in the high temperature anaerobic experiments with cattle

manure and stalks and is shown in *Figure 1*. The whole system was composed of two 1-L round wide-mouth bottles and one 1-L volumetric bottle, which were used for the apparatus for the digestion reaction of kitchen waste, the apparatus for the biogas collection, and the apparatus for water drainage, respectively. All apparatuses were connected with rubber tubes with anti-aging treatment to form a set of air-tight apparatuses. Air-tightness should be guaranteed in the connections. pH of the digested liquor was determined with the digital acidometer with accuracy of  $\pm 0.01$ . The composition of biogas was determined by gas chromatography (Shimadzu in Japan, GC-4B).

**Table 1.** Main parameters of the wet basic state of chicken manure, stalks, and active mud

Parameters	TS/%	VS/%	pH	TC/%	TN/%
Chicken manure	27.29	23.33	6.33	46.07	4.73
Stalks	91.44	86.50	6.89	50.02	0.88
Active mud	18.12	8.36	7.41	--	--



1-controller, 2-heating rod, 3-water bath, 4-sample reactor, 5-gas bottle, 6-gas bottle, 7-discharging bottle, 8-gas tube, 9-determination tube, 10-thermometer

**Figure 1.** The experimental apparatus for high temperature anaerobic digestion

### Experimental scheme

The methane production experiment with chicken manure and stalks used 24 1-L air-tight wide-mouth bottles as anaerobic digestion reactors and was conducted as eight groups with three replicates for each group. The average of the three experiments was reported. Except the experiment with R0 group, which received only 300 ml of inoculated mud, which was acclimatized without any other sample, the experiments with other seven groups received 60 g samples besides the inoculum of the same concentration and volume. The material ratios for high temperature anaerobic digestion of chicken manure and stalks (m/m, %) are shown in *Table 2*.

The addition of a 50 g sample in the experiment mainly considered the volume loading of the digester. Organic acids produced by decomposition of too much material would decrease pH of the digested liquor. The resultant acid inhibition would influence the production of biogas. The volume of 1 L was set with distilled water in experiments with 8 groups. Reactors were sealed up with glass cement and put into electric-heated

thermostatic water bath for constant cultivation for 50 d at 55 °C. The digested liquor was extracted with injector and put back into the reactor after pH determination with the digital acidometer with the determination frequency of one time per day. When pH values were below 6.8-7.2, NaHCO<sub>3</sub> was chosen as the regulation agent to maintain pH of the digested liquor above 6.8.

**Table 2.** The material ratios for high temperature anaerobic digestion of chicken manure and stalks (m/m, %)

	R1	R2	R3	R4	R5	R6	R7
Chicken manure	100	97	95	90	80	70	0
Stalk	0	3	5	10	20	30	100

### **Analytic method**

#### **Measurement of COD concentration**

In a 250 mL conical beaker, 10 mL of potassium dichromate was pipetted and 100 ml of distilled water was added. Subsequently, 30 ml of concentrated sulfuric acid was incorporated using the measuring cylinder. After cooling, 3 drops of the ferrous iron indicator was added and titrated with ammonium ferrous sulfate standard solution until the yellow solution changed into bluish-green and finally to reddish-brown. All test materials were set up in three parallel test groups (Zhang et al., 2017).

#### **Volatile fatty acids (VFAs)**

Volatile fatty acid content was determined by detecting volatile organic acid (C1-C5) using gas chromatography. The column size was HP-5, 30 m × 0.25 mm × 0.25 μm, and a flame ionization detector was used. Nitrogen was used as the carrier gas, and the flow rate was kept at 50 mL/min. The injector temperature was set at 300 °C, and the detector temperature was 230 °C. The sample was filtered through a 0.45 μm filter. From the filtered sample, 1 mL was taken and the pH was adjusted using formic acid (6 mol/L) to ensure pH < 6 for chromatographic determination (Hu et al., 2018).

### **Data processing**

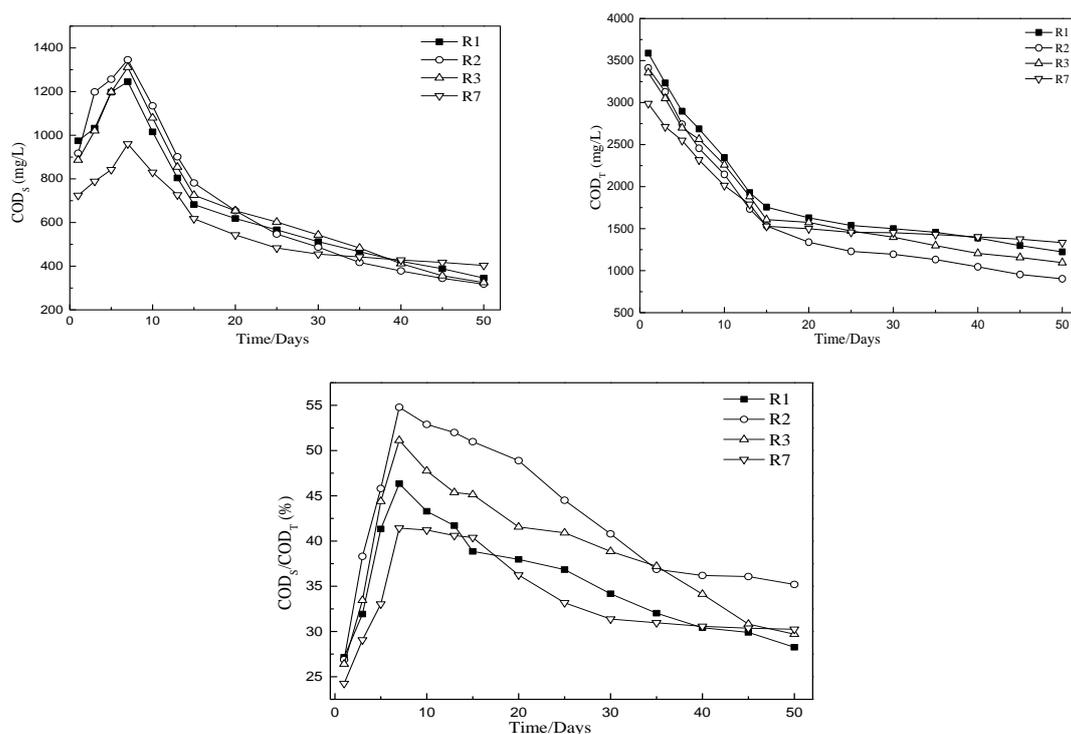
All the physical and chemical indicators in the tests were measured by setting three parallel tests. Data were tested for significance (p = 0.05) and correlation was analyzed using one-way ANOVA and multiple comparisons using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). All data plots were drawn using Origin-8.0. The differences between treatments and physical and chemical indicators during the test were analyzed using least significance difference (p < 0.05) (Naranjo et al., 2011).

## **Results and discussion**

### **COD concentration change during high-temperature anaerobic digestion using a sequencing batch reactor**

Figure 2 shows the trends of COD<sub>S</sub>, COD<sub>T</sub> and COD<sub>S</sub>/COD<sub>T</sub> during the sequential batch high-temperature anaerobic digestion of representative chicken manure and straw

in R1, R2, R3, and R7 groups. In the initial stage (1 d), the values of  $COD_T$  in R1, R2, and R3 were 3584.45-3356.45 mg/L, while the value of  $COD_T$  in R7 pure straw batch high-temperature anaerobic digestion was lower, i.e., 2987.39 mg/L. The reason is that the base of R7 is pure straw. Compared to R1 that contained pure chicken manure, R2 and R3 contained 97 and 95% chicken manure base, respectively. Straw is rich in the cellulose-hemicellulose-lignin component. Owing to the dense crystalline structure of cellulose, hemicellulose and lignin, these materials were difficult to break down. Therefore, the  $COD_T$  value of R7 was lower than the other three groups of base. During the 50-day high-temperature digestion process, the  $COD_T$  of the fermentation broth of the four groups showed a decreasing trend as a whole. This can be attributed to the high-temperature conditions during the process, in which the bacteria of hydrolysis acidification degraded the fermentation substrate to provide nutrient intermediates for the subsequent physiological metabolism of methanogenic microorganisms.



**Figure 2.** Trends of  $COD_s$ ,  $COD_T$  and  $COD_s/COD_T$  during sequential batch high-temperature anaerobic digestion of chicken manure and straw

The methanogenic microorganisms used the organic acid produced in the acidification stage to produce biogas, which led to a general decline in the  $COD_T$  of the fermentation broth of the four groups of tests. The degradation rates of  $COD_{T15}$  at 1-15 d were 51.06, 55.09, 52.11 and 48.84%. These rates were much higher than those in later days (16-50 d), which were 14.86, 18.44, 15.25 and 6.52%. This was mainly because the first 15 days of the initial reaction was the fastest stage of the high-temperature anaerobic digestion process. At this stage, the organic matter in the four experimental groups was mostly starch and protein, which are easy to break down. The high-temperature condition also facilitated hydrolysis and acidification, producing degradable organic matter such as monose, fatty acids and amino acids, causing pH to

decrease. The decrease in pH further promoted hydrolysis and acidification of refractory organic matter in the base. On the seventh day, the pH of the digestive juice was adjusted by adding 4 g sodium bicarbonate ( $\text{NaHCO}_3$ ) as the regulator to keep the pH above 6.8. After adding sodium bicarbonate ( $\text{NaHCO}_3$ ), the increased pH of the four groups of biogas slurry promoted methanogens to convert organic acids into methane and carbon dioxide. As the reaction finished (50 d), the  $\text{COD}_T$  values of the four groups were 1223.10, 903.21, 1095.39 and 1333.46 mg/L, respectively. The degradation rates of  $\text{COD}_{T50}$  were 65.92, 73.53, 67.36 and 55.36%.

Two main reasons account for the lower degradation rate of  $\text{COD}_T$  in the later stage. The first was the change of the base component of the high-temperature digestive juice. The degradable organic matter was rapidly broken down by methanogens under high-temperature conditions. The residual organic matter was mainly refractory organic matter, and the main component was cellulose-hemicellulose-lignin with a dense physical structure. It is difficult for the enzyme molecule and the water molecule to enter the interior and cause a hydrolysis reaction. Such substance is biodegradable, but the process takes a long time. The second was that the biochemical reaction of the fermentation broth was slowed down at the later stage due to the accumulation of metabolites. It was found that in the high-temperature anaerobic fermentation process of chicken manure and straw, the hydrolysis and acidification rate of organic matter remarkably increased at 55 °C. This led to the formation of more metabolites during the physiological metabolism process. The accumulation of metabolites had a certain inhibitory effect on the reproduction and metabolism of acid-producing methanogens in the later stage. Therefore, the degradation rate of  $\text{COD}_T$  in the later stage showed a downward trend, which was much lower than the degradation rate of  $\text{COD}_T$  in the first 15 days.

The value of  $\text{COD}_S$  at the initial stage of high-temperature anaerobic digestion solution for R7 was 724.39 mg/L, which was lower than the  $\text{COD}_S$  values of R1, R2 and R3 (974.25-886.33 mg/L). The reason is that in the R7 base, the content of straw was high and the content of starch and protein in straw was low. Since the cellulose-hemicellulose-lignin component is naturally difficult to hydrolyze, the content of insoluble organic matter was high, resulting in the lower  $\text{COD}_S$  of R7 high-temperature anaerobic digestion liquid at the initial stage (1 d). As the high-temperature digestion proceeded, the  $\text{COD}_S$  values of the four groups of high-temperature anaerobic digestion liquids for R1, R2, R3 and R7 all increased and reached the peak when the reaction proceeded to the seventh day. The values of the four groups of  $\text{COD}_S$  were 1245.33, 1345.69, 1311.28 and 959.89 mg/L, respectively. This is because the organic matter in the base of the four groups of fermentation broth was decomposed by the hydrolysis and acidification bacteria, and the insoluble macromolecular organic matter was hydrolyzed and acidified into small organic acids. The decrease in pH further facilitated the hydrolysis and acidification of the refractory organic matter, and dissolved in water, leading to an increase in the  $\text{COD}_S$  of the four groups of fermentation broth (den Boer et al., 2012). As the reaction progressed, the  $\text{COD}_S$  showed a declining trend for two reasons. The first is that the dissolved COD was utilized by methanogens to convert to carbon dioxide and methane. The second is that the relative content of dissolved COD was reduced as the concentration of total organic matter decreased. Therefore, the  $\text{COD}_S$  showed a downward trend in the later stage. At the end of the reaction (50 d), the values of the four groups of  $\text{COD}_S$  were 345.66, 317.89, 325.33 and 403.28 mg/L, respectively.

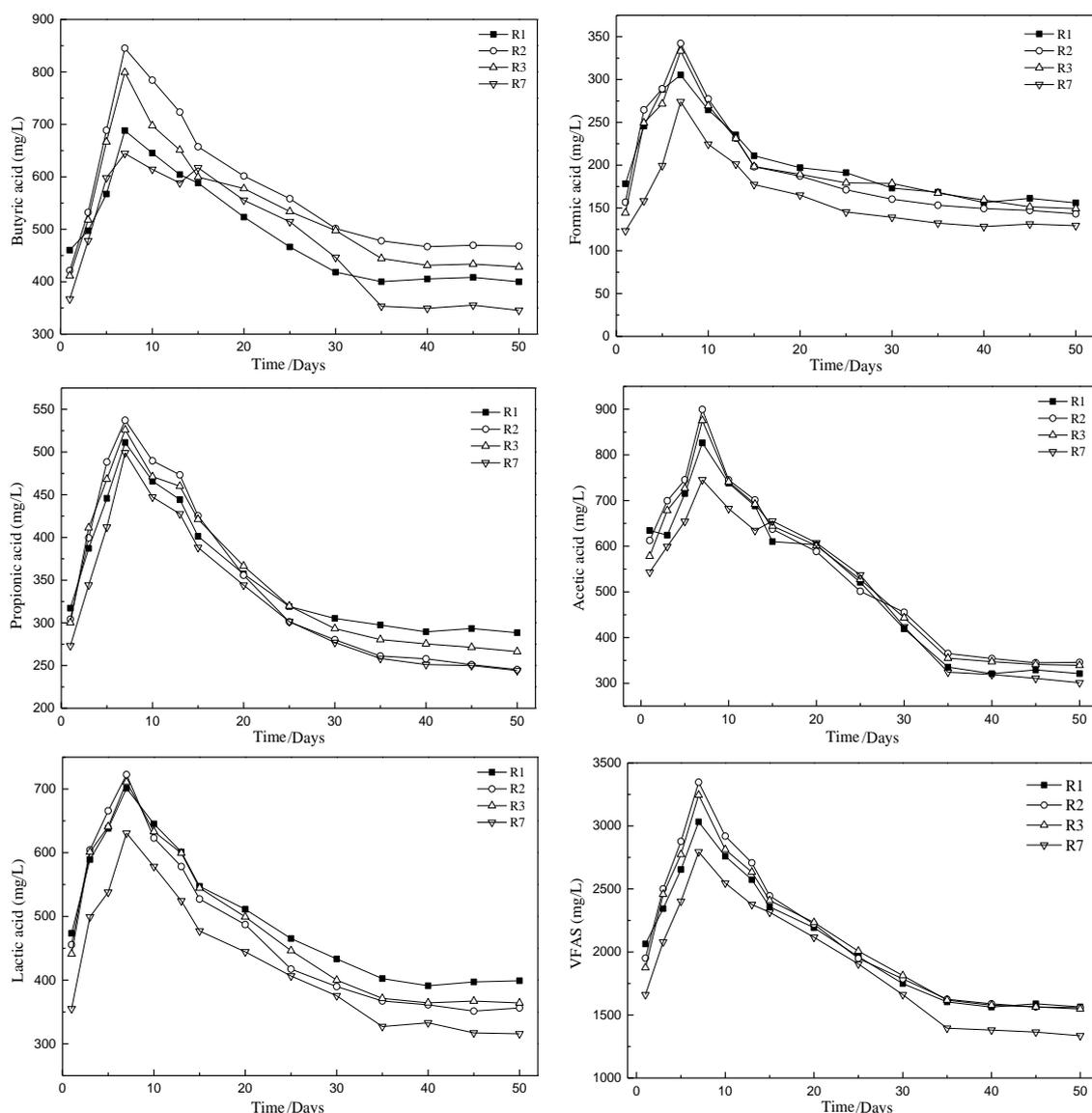
With the high-temperature digestion reaction, the ratio of  $COD_S/COD_T$  in the high-temperature anaerobic digestion liquid of R1, R2, R3 and R7 all increased. During this period, the organic matter in the fermentation liquid was hydrolyzed and acidified, and the macromolecule was insoluble in water, which led to an increase in the CODs concentration. However, the  $COD_T$  concentration decreased as the reaction progressed, and thus the ratio of  $COD_S/COD_T$  increased. The reaction reached the peak on the seventh day, and the  $COD_S/COD_T$  ratios of the four groups of fermentation broth were 46.34, 54.78, 51.14 and 41.43%, respectively. The  $COD_S/COD_T$  ratio decreased with the reaction because the methanogens decomposed CODs, which hastened the decreasing rate of dissolved COD and thus led to a decrease in the  $COD_S/COD_T$  ratio. At the end of the reaction (50 d), the  $COD_S/COD_T$  ratios of the four groups were 28.26, 35.20, 29.70, and 30.24%, respectively.

### ***Correlation study between VFAs and each component in sequential batch high-temperature anaerobic digestion***

Figure 3 shows the variation trend of VFAs and concentration of each component in sequential batch high-temperature anaerobic digestion of chicken manure and straw. In this section, four representative experimental data of R1, R2, R3 and R7 were selected for analysis. The values of VFAs in the initial stage (1 d) were 2063.84, 1950.4, 1875.32 and 1661.66 mg/L, respectively. In total VFAs, the fraction of formic acid, acetic acid, propionic acid, lactic acid and butyric acid were R1: 8.64, 30.75, 15.37, 22.94 and 22.30%, respectively; R2: 8.03, 31.41, 15.60, 23.36 and 21.60%, respectively; R3: 7.69, 30.84, 16.01, 23.52 and 21.94%, respectively; and R7: 7.42, 32.69, 16.44, 21.36 and 22.08%, respectively. As the high-temperature anaerobic digestion proceeded, formic acid, acetic acid, propionic acid, lactic acid, butyric acid, and total VFAs all increased. The values reached the maximum peak on the seventh day of the reaction. The VFAs values of R1, R2, R3 and R7 were 3032.39, 3346.75, 3455.12 and 2794.03 mg/L, respectively. Among these digestive juices, the contents of formic acid, acetic acid, propionic acid, lactic acid and butyric acid were 9.81-10.27%, 26.28-27.28%, 16.05-17.87%, 21.59-23.13%, and 22.70-25.26%, respectively. The stage when pH decreased corresponded to the stage when VFAs concentration increased, while the stage when pH increased corresponded to the stage when VFAs concentration decreased. The pH in the pH reaction system had a corresponding relationship with the change of volatile acid content, i.e., the pH value decreased while the VFA concentration increased, and the VFA concentration reached the maximum value as the pH value reached the minimum; the pH value increased while the VFA concentration decreased. In the test and actual production, the change in the pH value can reflect the value of VFA concentration, controlling the acidizing process macroscopically (Duan et al., 2016). Subsequently, the VFAs of the digestive juices of the four experimental groups decreased. When the reaction was completed (50 d), the VFAs of the experimental digestive fluids of R1, R2, R3 and R7 were 1564.6, 1558.34, 1547.37 and 1335.58 mg/L, respectively. The contents of formic acid, acetic acid, propionic acid, lactic acid, and butyric acid were 9.19-9.97%, 20.58-22.13%, 15.74-18.44%, 22.86-25.50% and 25.56-30.02%, respectively.

The concentration of VFAs during the sequential batch high-temperature anaerobic digestion of chicken manure and straw was determined by the rate of production and consumption of formic acid, acetic acid, propionic acid, butyric acid and lactic acid. As the high-temperature anaerobic digestion proceeded, formic acid, acetic acid, propionic

acid, lactic acid, butyric acid and total VFAs all increased. On the seventh day of the reaction, the presence of peak value was due to the use of base. For R1, R2 and R3, the content of chicken manure was high, of which the degradable organic matter was converted to small organic acid. However, at this stage, the methanogens were still in the period of adaptation, and the biological metabolic activity was not strong. The enzymatic reaction rate was slow, and the production rate of VFAs was greater than the metabolic rate, resulting in a decrease in the pH of the system (Yang et al., 2017). The pH drop further inhibited the activity of the methanogens, leading to the maximum of VFAs concentration on the seventh day, i.e., 3032.39, 3346.75 and 3245.12 mg/L, which showed the highest content of acetic acid, lactic acid and butyric acid. In the R4 experiment, pure straw was used as the base material, and the dense structure of the straw made hydrolysis reaction difficult (Zhou et al., 2016, 2018, 2019). Therefore, the produced content of small organic acid was relatively low, and the VFAs concentration reached 1335.58 mg/L on the seventh day.



**Figure 3.** Trends of VFAs and concentration of various components during sequential batch high temperature anaerobic digestion of chicken manure and straw

The main reason for the decline of VFAs in the digestive juices of the later four groups was that the pH of the digestive juice was adjusted by adding sodium bicarbonate ( $\text{NaHCO}_3$ ) on the seventh day (Zhang et al., 2011). The methanogens in anaerobic digestion then began to adapt, and the suitable pH value also increased the anaerobic biochemical enzymatic reaction rate. This promoted the degradation of acetic acid, lactic acid and butyric acid-based VFAs, and led to the further increase in pH. When the reaction was completed (50 d), the values of VFAs in the digestive juice of R1, R2 R3 and R7 were 1564.6, 1558.34, 1547.37 and 1335.58 mg/L, respectively. Among these digestive juices, the contents of formic acid, acetic acid, propionic acid, lactic acid and butyric acid were 9.19-9.97%, 20.58-22.13% and 15.74-18.44%, 22.86-25.50% and 25.56-30.02%, respectively.

## Conclusions

(1) The  $\text{COD}_T$  of the fermentation broth of R1, R2, R3 and R7 showed a downward trend. The  $\text{COD}_T$  of the fermentation broth showed a significant downward trend in the first 15 days, and  $\text{COD}_T$  values decreased from 3584.45, 3132.28, 3355.45 and 2987.39 mg/L at the initial stage to 1756.28, 1532.45, 1607.28 and 1528.33 mg/L. The degradation rates of  $\text{COD}_T$  15 were 51.06, 55.09, 52.11 and 48.84%. At the end of the reaction (50 d), the values of the four groups of  $\text{COD}_T$  were 1223.10, 903.21, 1095.39 and 1333.46 mg/L, and the degradation rates of  $\text{COD}_T$ 50 were 65.92, 73.53, 67.36 and 55.36%.

(2) The VFAs values in the initial stage of reaction (1d) of R1, R2, R3 and R4 were 2063.84, 1950.4, 1875.32 and 1166.66 mg/L, respectively. As the high temperature anaerobic digestion proceeded, formic acid, acetic acid, propionic acid, lactic acid, butyric acid and total VFAs all increased. As the reaction proceeded to the seventh day, the maximum concentration, the VFAs of the digestive fluids from R1, R2, R3 and R7, were 3032.39, 3346.75, 3245.12 and 2794.03 mg/L, respectively. At the end of the reaction (50 d), the VFAs of the four groups of experimental digestive juices were 1564.6, 1558.34, 1547.37, and 1335.58 mg/L, respectively. Among these digestive juices, the contents of formic acid, acetic acid, propionic acid, lactic acid, and butyric acid were 9.19-9.97%, 20.58-22.13%, 15.74-18.44%, 22.86-25.50% and 25.56-30.02%, respectively.

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