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Full Length Article

A Study of COD Degradation by High Temperature Dry Fermentation of Chicken Manure and Straw

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Abstract

Dry fermentation has become a hot spot due to characteristics as low water requirements, high gas production rates, and low costs. In this work, high temperature dry fermentation of chicken manure and straw was conducted at 55±0.2°C to analyze the changes of COD_T, COD_S, and the ammonium nitrogen concentration as well as their relationships. It was shown that according to the changes of the loading frequency, the biogas production rate, pH, COD_T, COD_S, and the ammonium nitrogen concentration, the whole experimental process of high temperature dry fermentation of chicken manure and straw was divided into four periods such as the stagnant adaptation period (20 d, from 1–20 d), the quick start period (30 d, from 21–50 d), the stable period (70 d, from 51–120 d), and the overload period (20 d, from 121–140 d). During the stable period (70 d, from 51–120 d), COD_T concentration in the high temperature dry fermentation digestive liquor in reactors R1–R4 increased with the increase of the loading frequency from 13.45, 15.32, 16.11, and 18.11 g/L on the 51st d to 17.89, 20.32, 22.65, and 25.87 g/L on the 120th d, respectively. The proportion of COD_S to COD_T in the fermentation liquor in R1–4 reactors increased and was maintained at 45.65–47.21, 51.01–55.32, 44.12–48.82, and 32.01–39.15%, respectively. The ammonium nitrogen concentration increased to 2688.91 ± 68.35, 2479.45 ± 244.36, 2307.01 ± 175.36, and 1445.22 ± 88.32 mg/L on the 120th d, respectively, with the increase of the loading frequency. © 2020 Friends Science Publishers

Keywords: Chicken manure; COD; High temperature dry fermentation; Mechanism study; Straw

Introduction

Compared to anaerobic digestion or wet fermentation, dry fermentation has the characteristics as less water consumption, high biogas production rates, low costs, *etc*. Nevertheless, dry fermentation is difficult to start and the parameters of the process are difficult to adjust (Linke 2006; Chu *et al.* 2010; Li *et al.* 2016). Moreover, concentrated ammonia and toxic metabolites inhibit the fermentation process. These drawbacks limit popularization of the dry fermentation technology. In the past two years, encouraged by valorization of biomass resources to avoid the energy crisis, the dry fermentation technique for organic waste attracted more attention due to its advantages. In Western European countries, such as Germany, the dry fermentation technology for biogas production from domestic waste has been developed, especially for market-oriented applications (Chen *et al.* 2008; Zhang and Jahng 2012; Duan *et al.* 2016). In 2000, the dry fermentation capacity of European domestic waste accounted for 54% of the total fermentation capacity (Tauseef *et al.* 2013). From 2006 to 2011, the proportion of dry fermentation in newly built anaerobic fermentation units for domestic waste further increased to 63% (Nasir *et al.* 2012). To date, dry fermentation is prevalently conducted at medium temperatures and the feedstock is mainly kitchen waste. Few reports have been reported on the high-temperature dry fermentation technology using livestock and poultry manure, chicken manure, and straw. The commercial applications of dry anaerobic fermentation mainly consume domestic waste instead of livestock and poultry manure. This is because the nitrogen content in livestock and poultry manure is very high and suppresses fermentation, and ammonia very easily accumulates in the dry fermentation process. Therefore, in this paper, straw was added to regulate the C/N ratio in the high-temperature dry fermentation of livestock and poultry manure to shorten HRT and improve the total solid content (TS) in the fermentation liquid to higher than 20%. This technique targets direct feeding and discharging and stable biogas production. The high-temperature fermentation of chicken manure and straw represents a viable technical scheme for valorization of livestock and poultry manure and straw.

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In recent years, experts and scholars at home and abroad have studied the fermentation of domestic waste (Feng *et al.* 2020) and animal manure such as cattle, pigs, chickens, *etc*. Lei Feng and others have carried out dry fermentation of food waste. It has been found that under different environmental conditions, the concentration of volatile fatty acids (VFAs), cod and ammonia nitrogen has changed significantly (Feng *et al.* 2019c). According to the research of Xiaofei Zhen and others, the C/N ratio of the mixture of food waste and urban sludge is not the same in the same way, the effect on the growth and metabolism of microorganisms in the fermentation process is also different (Zhen *et al.* 2019). Used cow manure, pig manure, chicken manure and 10% corn straw as the fermentation agent for aerobic static composting test. The results showed that in the whole composting process, the activity of urease and invertase was basically the same, and the overall trend was declining, and the content of total nitrogen, total phosphorus and total potassium was increasing (Feng *et al.* 2019a, 2019b). However, due to the high nitrogen content in livestock manure, ammonia is easy to accumulate in the whole fermentation process. Based on this, in the process of high-temperature dry fermentation of livestock and poultry manure, adding straw to regulate the C/N ratio, shorten hydraulic retention time (HRT) and increase the total solid content (TS) of fermentation liquid to more than 20%, which provides a feasible technical scheme for the price of livestock and poultry manure and straw.

Materials and Methods

Chicken manure in the experiment came from a chicken farm in Beipiao Country, Chaoyang City, Liaoning Province. After careful screening to remove impurities, such as stone, chicken manure collected for continuous 3 d was sent to the laboratory directly and kept at 4°C in refrigerator (Jiménez *et al.* 2003; Yeoung 2005). 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. 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. 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 (Rundberget *et al.* 2004; Zhang *et al.* 2017; Wang *et al.* 2019), 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 (Qin *et al.* 2011; Hu *et al.* 2018). 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 (Boer *et al.* 2012). The organic content was determined after 4 h heating in the muffle furnace at constant 550°C (Duan *et al.* 2016). Main parameters of the wet basic state of chicken manure, stalks, and active mud are shown in Table 1.

The high-temperature dry fermentation using chicken manure and straw was carried out on the basis of the previous experiment. A 4 × 30-liter anaerobic bioreactor (Baoxing, Shanghai) was used. The reactor content was stirred regularly through a central controller. Based on the results of the previous experiment, the feedstock with straw mass ratios of 0, 3, 5, and 100% was prepared. During the start-up stage of high-temperature dry fermentation, 3 L of anaerobic sludge for inoculation, cultivated in Shenyang North Sewage Treatment Plant, was mixed with 3 kg of the feedstock, and then the mixture was placed into the feeding tank of the bioreactor, which was equipped with an air inlet and outlet. N_2 was introduced for 5 min. After oxygen was discharged from the outlet, the feeding valve was opened for the addition of feedstock into the bioreactor. In the experiment, fresh feedstock was added from the top of the bioreactor after spent materials were discharged from the bottom of the bioreactor. For fermentation, the volume of materials was set to 20 L with water, and the temperature was adjusted to 55 ± 0.2°C (García-Ochoa *et al.* 1999). During the experiment, $NaHCO_3$ was added to maintain pH of the digestion liquid above 6.8. When the system reached a stable state, hydraulic retention time (HRT) was shortened from 200 to 20 days to increase the total solid content (TS) to higher than 20%. Direct feeding and discharging and stable biogas production continued until the high-temperature dry fermentation was completed. In the last stage of the experiment, HRT was further shortened to 10 days for a destructive experiment to study the inhibition effect of the high TS content on the dry fermentation process. In the experiment, the biogas production rate, pH, VFAs concentrations, and other parameters of the system were monitored, and the changes and relations of these parameters were analyzed. All the experiments were conducted twice, and the average values were adopted as valid data.

Results

Results of COD and ammonium nitrogen concentration changes in high temperature dry fermentation

Fig. 1 to 4 show the pH, ammonium nitrogen and total chemical oxygen demand (COD_T) concentration changes in

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	--	--

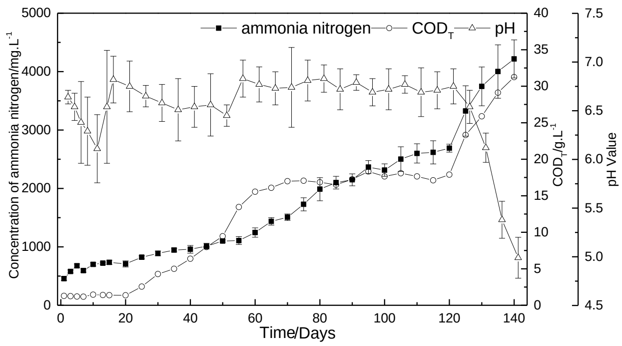

Fig. 1: The changes of pH, the ammonia nitrogen and COD_T concentration in R1 reaction

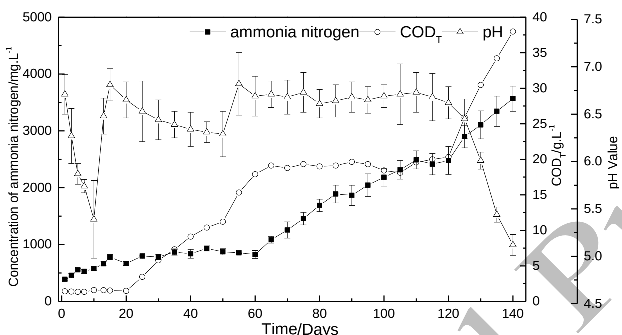

Fig. 2: The changes of pH, the ammonia nitrogen and COD_T concentration in R2 reaction

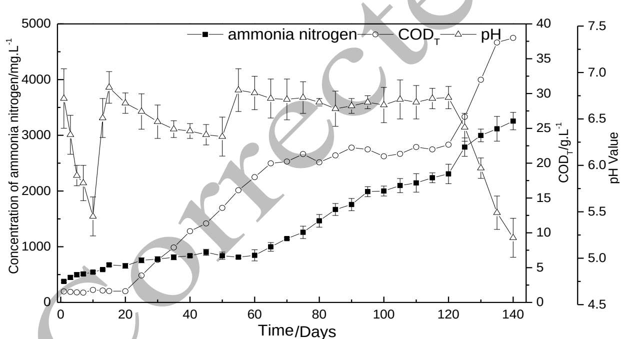

Fig. 3: The changes of pH, the ammonia nitrogen and COD_T concentration in R3 reaction

the process of high temperature dry fermentation of chicken manure and straw. According to the loading frequency, the whole experimental process of high temperature dry fermentation of chicken manure and straw was divided into four periods, namely the stagnant adaptation period (20 d, from 1–20 d), the quick start period (30 d, from 21–50 d),

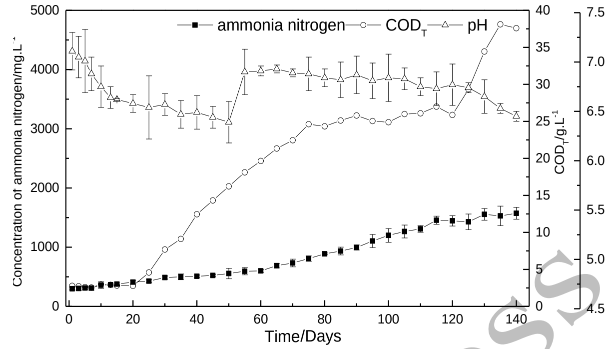

Fig. 4: The changes of pH, the ammonia nitrogen and COD_T concentration in R4 reaction

the stable period (70 d, from 51–120 d), and the overload period (20 d, from 121–140 d). In four reactors, pH, total COD (COD_T) concentration, and the ammonium concentration in the fermentation liquor were correlated with the TS concentration. During the whole 140-d high temperature dry fermentation, both concentrations increased with the increase of the loading frequency.

In the stagnant adaptation period (20 d, from 1–20 d), there was only material input and no material output. 1 kg materials were added every ten days. COD_T concentration in the digestive liquor on the first day of high temperature dry fermentation in 4 reactors was 1.29, 1.42, 1.58 and 2.78 g/L, respectively, and the proportion of soluble COD (COD_S) concentration to COD_T concentration was 29.89, 27.45, 26.01, and 17.21%, respectively. Besides, the initial ammonium nitrogen concentrations were 455.38 ± 32.25, 388.77 ± 16.35, 377.43 ± 19.88, and 298.46 ± 17.65 mg/L, respectively. During ten days from the first loading (1–10 d), COD_T concentration in R1–4 demonstrated a trend of decrease and decreased to 1.18, 1.34, 1.41, and 2.54 g/L on the 10th d, respectively, and the proportion of COD_S to COD_T concentration decreased to 21.00, 22.04, 22.18, and 14.11%, respectively. Besides, the ammonium nitrogen concentrations increased to 594.21 ± 28.65, 528.39 ± 36.65, 511.07 ± 24.98, and 309.45 ± 33.65 mg/L, respectively. During the stagnant adaptation period, the decrease of COD_T concentration in R1–4 was due to two loadings, namely one loading per 10 days. The physical and chemical principles of this dry fermentation reaction within 1–10 d and 11–20 d were consistent with those of the short previous reaction. With the process of biological degradation, organic matter was decomposed. Some of organic acids were utilized by methanogens to produce biogas *via* anaerobic digestion, leading to the decrease of total COD concentration in the fermentation liquor. There were two reasons for the decrease of the proportion of COD_S to COD_T concentration in the fermentation liquor during the stagnant adaptation period. On the one hand, with the process of dry fermentation, decomposition of easily degradable organic matter resulted in the decrease of COD_S concentration in the fermentation liquor. Meantime, the proportion of COD_S to

COD_T concentration demonstrated a trend of decrease. On the other hand, due to slow adaptation of microorganisms to the system during this period, anaerobic digestive microorganisms could not grow and reproduce immediately. They required an adaptation time to generate the enzyme system for metabolic nutrition and further growth and reproduction. Thus, hydrolytic acidification was slow in this period. Organic matter in total COD could not be effectively transformed to soluble COD. Thus, the proportion of COD_S to COD_T concentration in the fermentation liquor decreased in this stagnant adaptation period. The proportion of COD_S to COD_T concentration on the 20^{th} d decreased to 24.55, 26.14, 25.12, and 16.01%, respectively. The continuous increase of the ammonia nitrogen concentration in the whole process was due to the C/N/P ratio of 200:5:1 required by anaerobic microorganisms in the traditional anaerobic digestion process.

During the biogas production by high temperature anaerobic fermentation of chicken manure and straw, a large amount of organic carbon was transformed to CH_4 and CO_2 by anaerobic microorganisms. However, N was not basically utilized except for physiological metabolism of microorganisms. Thus, the ammonia nitrogen concentration in the fermentation liquor demonstrated an increasing trend in the whole stagnant adaptation period. The ammonia nitrogen concentration increased to 708.46 ± 52.24, 666.68 ± 35.26, 655.28 ± 44.18, and 411.05 ± 31.11 mg/L, respectively. In the quick start period (30 d, from 21–50 d), the loading frequency was increased. 1 kg materials were added every 3 and 2 days during 21–40 d and 41–50 d, respectively.

COD_T concentrations in the digestion liquor in R1–4 reactors of high temperature dry fermentation increased with the increase of the loading frequency. COD_T proportions on the 50^{th} d in R1–4 reactors were 9.45, 11.21, 13.58, and 16.22 g/L, respectively. The proportions of COD_S to COD_T concentrations in the fermentation liquor increased from 31.22, 33.45, 31.45, and 23.21% on the 21^{st} day to 43.35, 49.21, 44.35, and 30.05% on the 50^{th} d. The ammonia concentration increased from 822.56 ± 26.65, 798.74 ± 15.32, 756.38 ± 45.65, and 427.08 ± 25.32 mg/L on the 21^{st} day to 1100.19 ± 18.22, 872.38 ± 55.37, 838.28 ± 66.12, and 555.39 ± 88.77 mg/L on the 50^{th} d, respectively. During the quick start period, COD_T concentration in the digestion liquor in R1–3 and 7 reactors of high temperature dry fermentation increased with the increase of the loading frequency due to the increase of TS concentration in the reactors with the increase of the loading frequency. Organic matter content increased proportionally, leading to the increase of TCOD concentration in the system.

The result of mechanism of high temperature dry fermentation of chicken manure and straw

Based on the four stages theory of anaerobic digestion, experimental results of R2 were chosen to analyze the mechanism of high temperature dry fermentation of chicken manure and straw stage by stage, comprehensively considering the gas production, COD concentration, and the ammonium nitrogen concentration. According to changes of the loading frequency, the biogas production rate, COD concentration, and the ammonia nitrogen concentration in the high temperature dry fermentation of chicken manure and 0.3% straw, the whole experimental process could be divided into four stages, namely the stagnant adaptation period (20 d, from 1–20 d), the quick start period (30 d, from 21–50 d), the stable period (70 d, from 51–120 d), and the overload period (20 d, from 121–140 d).

The stagnant adaptation period (20 d, from 1–20 d): During the stagnant adaptation period, there was no material output but only material input with 1 kg material every 10 days. HRT (1–20 d) was 200 d. The loading material and the regulator (10 g of $NaHCO_3$) were simultaneously added every time to prevent inhibition of the reaction by excessive acidification. The biological and chemical reaction principles were consistent with those of the short term previous reaction. TS concentrations, pH, and COD_T concentrations in the reactors demonstrated a decreasing trend. The biogas production rate was relatively lower, whereas the biological degradation rate was much higher, namely 62.41%. There should be an adaptation process to apply 3 L of anaerobically activated sludge, which was acclimated in the stagnant adaptation period to the high temperature anaerobic digestion system. Thus, HRT (1–20 d) was much longer. In the anaerobic digestion process, as in the adaptation period of the previous experiment, anaerobic digestive microorganisms could not grow and reproduce immediately. During the period of adaptation, they hardly grew and reproduced while the enzyme system for metabolic nutrients was built.

In the late period of the stagnant adaptation stage, the quantity of anaerobic digestive microorganisms increased, promoting the increase of the biogas production rate. The main mechanism in this stage was that the flora for hydrolysis and fermentation adapted to the high temperature dry fermentation system and generated the enzyme system for metabolism of nutrients from chicken manure and straw. Organic matter in chicken manure and straw, such as starch and a small part of cellulose, was hydrolyzed to small organic acids and monosaccharides. Proteins were also hydrolyzed to amino acids, which gave organic acids and ammonia by deamination. Some of lipids were hydrolyzed to lower fatty acids and alcohols, such as acetic acid, propanoic acid, butanoic acid, long chain fatty acids, ethanol, CO_2, H_2, NH_3, and sulfides *etc*., which decreased the system pH. The decrease of pH inhibited the activity of methanogens. Thus, in the late phase of the stagnant adaptation period, only some of soluble small organic molecules entered the microorganisms by active transportation and were utilized by anaerobic methanogens to produce CH_4 and CO_2. Due to faster acidification compared with methanation, CH_4 concentration in biogas during this stage was not very high and maintained around 43.75%. Thus, HRT (1–20 d) was relatively longer, whereas the biological degradation rate was high, namely 62.41%.

The quick start period (30 d, from 21–50 d): There was only material input and no material output due to the increased loading frequency from the 21st d of the reaction. 1 kg material was added every 3 and 2 days during periods of 21–40 d and 41–50 d, respectively. HRT (21–40 d) and HRT (41–50 d) were 60 d and 40 d, respectively. This stage was semi continuous anaerobic digestion. The TS concentration, biogas production rate, COD_T concentration, the proportion of COD_S to COD_T concentration, and the ammonia nitrogen concentration all increased with the increase of the loading frequency of R2 reactor. There was no addition of $NaHCO_3$ to adjust pH of the digestion liquor during the loading in this stage. Thus, the pH in R2 of high temperature dry fermentation of chicken manure and straw demonstrated a decreasing trend. Thus, HRT decreased in this stage, and the biological degradation rate decreased to 57.33%. After 20 days of the stagnant adaptation period, not only hydrolytic acidogenic flora as well as methanogens but also the metabolic activities increased dramatically. With the increase of the loading frequency, the TS concentration increased. COD concentration, especially soluble COD concentration, increased due to the increase of substrates, which could be hydrolyzed and acidified. The reason was that proteins, starch, and cellulose were hydrolyzed by hydrolytic acidogenic microorganisms to produce organic acids, which were all organic acids with more than three carbon atoms, long chain fatty acids, aromatic acids, and alcohols *etc*. They could be decomposed further by bacteria producing H_2 and acetic acid and by sulfate reducing bacteria.

The dry fermentation of chicken manure and straw was conducted in one stable and single phase anaerobic digestive biological and chemical reactor with main metabolites acetic acid, CO_2, and H_2 by acidogenic microorganisms, whereas H_2 could be effectively utilized by methanogens as a reducing substrate. In the presence of methanogens and the lack of sulfates, the sulfate reducing bacteria could change ethanol and lactic acid to acetic acid, H_2, and CO_2. There was synergetic relation between methanogens and the bacteria reducing sulfates.

The stable stage (70 d, from 51–120 d): There were 1 kg material input and output every day from the 51st d. HRT (51–120 d) was 20 d. Continuous digestion was realized in this stage. The TS concentration exceeded 20% in this stage, illustrating the dry fermentation.

The TS concentration, biogas production rate, COD_T concentration, the proportion of COD_S to COD_T concentration, and the ammonia nitrogen concentration in R2 reactor increased with the increase of the loading frequency. HRT decreased in this stage, and the biological degradation rate decreased to 48.35%. There was balanced development of hydrolytic and acidogenic flora, H_2 and acetic acid producing flora, and specific anaerobic methanogens in this system in this stage. With the increase of the loading frequency, the continuous supply of organic matter to the reactors satisfied the requirements of hydrolytic and fermentation flora for nutrients. Organic matter in chicken manure and straw, such as starch and a small part of cellulose, was hydrolyzed to small organic acids and monosaccharides. Proteins were hydrolyzed to amino acids, which were transformed to organic acids and ammonia by deamination. Some of lipids were hydrolyzed to lower fatty acids and alcohols, such as acetic acid, propanoic acid, butanoic acid, long chain fatty acids, ethanol, CO_2, H_2, NH_3, and sulfides. Organic acids with more than three carbon atoms, long chain fatty acids, aromatic acids and alcohols *etc*. were further decomposed to generate acetic acid and H_2 by bacteria producing H_2 and acetic acid as well as by bacteria reducing sulfides. However, pH of the system was maintained above 6.8 after adjustment, which enhanced the metabolic activity of methanogens. The produced small organic acids were transformed to methane and CO_2, preventing the decrease of pH in the system.

The overload period (20 d, from 121–140 d): 2 kg material was added every day from the 121st d. HRT (121–140 d) was 10 d. The TS concentration, COD_T concentration, and the ammonia nitrogen concentration in R2 reactor increased with the increase of the loading frequency. However, the biogas production rate, the proportion of COD_S to COD_T concentration as well as pH decreased sharply. In the late period of the overload stage, system stirring and material output became difficult due to ground straw expansion in the material because of high temperature and water adsorption.

The gas production decreased in the system. Chicken manure and straw were discharged directly without biological and chemical degradation during material output. The biological degradation rate decreased to 22.35%. During the whole overload period, the hydrolysis and acidification were inhibited by the decreased water activity in the reactors caused by the excessive increase of TS concentration in the high temperature dry fermentation reactors because of the increased loading. Meantime, the easily degradable organic matter content was high. PH of the system decreased with small organic acids due to the metabolism of hydrolytic acidogenic microorganisms, which further inhibited the activity of methanogens and decreased the corresponding enzymatic reaction rate, leading to the unbalanced proportion of flora in the system as well as to unstable gas production and operation. Meantime, due to inhibition of the ammonia nitrogen, the ammonia nitrogen concentration increased to a level toxic for anaerobic digestive methanogens with the increase of the loading and TS concentration in the system with long term operation. The activity of methanogens was thus inhibited. Besides, stability and gas production ability of the system began to decrease.

Discussion

The increase of the proportion of COD_S to COD_T in the fermentation liquor was mainly due to enhanced adaptation of anaerobic digestive microorganisms to the high temperature digestion reaction. When the growth rate of microorganisms increased to the maximum, the quantity of hydrolytic acidogenic bacteria increased geometrically. The sharp increased microorganism quantity as well as the high metabolic activity resulted in transformation of insoluble organic matter and macromolecules of organic matter in TCOD to organic acids with soluble small molecules. COD_S concentration in the system increased. Thus, the proportion of COD_S to COD_T in the fermentation liquor increased in the quick start period. The increase of the ammonia nitrogen concentration in the fermentation liquor in R1–4 reactors during this period was the same as in the stagnant adaptation period because a large amount of organic carbon was transformed to CH_4 and CO_2 by anaerobic microorganisms in the biogas production by high temperature anaerobic fermentation of chicken manure and straw. N was basically seldom utilized except for physiological metabolism of microorganisms. Thus, the ammonium nitrogen concentration in the fermentation liquor demonstrated an increasing trend in the whole stagnant adaptation period.

The stable period (70 d, from 51–120 d) There were material input and output every day from the 51st d. With the increase of TS concentration, COD_T concentration in the digestion liquor in R1–4 reactors increased from 13.45, 15.32, 16.11, and 18.11 g/L on the 51st day to 17.89, 20.32, 22.65, and 25.87 g/L on the 120th day with the increase of the loading frequency.

The proportion of COD_S to COD_T in the fermentation liquor in R1–3 and 7 reactors in the stable period increased and maintained at 45.65–47.21, 51.01–55.32, 44.12–48.82, and 32.01–39.15%, respectively. The ammonia nitrogen concentration increased to 2688.91 ± 68.35, 2479.45 ± 244.36, 2307.01 ± 175.36, and 1445.22 ± 88.32 mg/L on the 120th d, respectively, with the increase of the loading frequency.

The reason for the increase of COD_T concentration in the digestion liquor in R1–4 reactors of high temperature dry fermentation during the stable period with the increase of the loading frequency was the same as in the quick start period. Material input and output were realized every day in this stage. With the increase of the loading frequency, the TS concentration in the reactors increased. Besides, the organic matter content increased proportionally, leading to the increase of TCOD concentration in the system, which finally increased to 17.89, 20.32, 22.65, and 25.87 g/L on the 120th d, respectively. In the stable period, the proportion of COD_S to COD_T in the fermentation liquor in R1–4 reactors increased due to complete adaptation of the bacterial flora of high temperature dry fermentation to the reaction system.

The quantity of microorganisms for hydrolysis and fermentation as well as the biological degradation ability reached the maximum. The internal bacterial flora of the system was balanced.

The hydrolytic acidification of organic matter by Zoogloea was the fastest, promoting the hydrolysis of insoluble macromolecules of organic matter and increasing the concentration of soluble COD. The increase of the ammonia nitrogen concentration with the increase of the loading frequency was due to higher requirements for organic carbon than for nitrogen by anaerobic acidogenic bacteria and methanogens in high temperature dry fermentation of chicken manure and straw. Organic carbon was needed for the generation of CO_2 and CH_4, whereas only a small part of N was utilized by flora during the physiological metabolism process. Thus, the ammonium nitrogen concentration on the 120th d reached 2688.91 ± 68.35, 2479.45 ± 244.36, 2307.01 ± 175.36, and 1445.22 ± 88.32 mg/L, respectively.

During the stable period, COD_T concentration in R4 reactor was higher than that in R1–3 reactors. Besides, the proportion of COD_S to COD_T in the fermentation liquor was lower than that in R1–3 reactors. Both were due to the pure straw substrate in R4. In high temperature dry fermentation, the easily degradable organic matter in the substrate was mainly cellulose or semi cellulose whose dense physical structure inhibited the entry of biological enzymes to the interior, which was unfavorable to the hydrolysis reaction.

The proportion of COD_S to COD_T in the fermentation liquor in R2 reactor was higher than that in R1–3 due to the increased 3% straw content in the substrate of R2. The increase of carbon content in the substrate adjusted the ratio of C/N, promoting the physiological metabolic activity of microorganisms in high temperature dry fermentation. The corresponding hydrolysis and acidification rate was estimated to be the highest. Besides, the organic acids concentration was relatively higher. Thus, the proportion of COD_S to COD_T in the fermentation liquor in R2 was the highest.

In the overload period (20 d, from 121–140 d), COD_T concentration in R1–4 reactors of the high temperature dry fermentation of chicken manure and straw increased dramatically with the increase of the added material quantity from 23.32, 25.88, 26.65, and 29.33 g/L on the 121st d to 31.25, 37.98, 37.99, and 37.59 g/L on the 140th d of the experiment. During this period, the proportions of COD_S to COD_T in the fermentation liquor in R1–4 reactors all decreased and maintained at 21.33–36.54%. However, the ammonium nitrogen concentration increased dramatically. On the 140th d of the overload period, the ammonia nitrogen concentration in the fermentation liquor in R1–3 and R7 reactors reached 4216.39 ± 325.32, 3566.28 ± 222.31, 3255.21 ± 156.32, and 1573.28 ± 99.98 mg/L, respectively.

The main reason for the dramatic increase of COD_T in R1–4 reactors of the high temperature dry fermentation of chicken manure and straw was due to the increase of daily quantity of material, which relatively increased the input

COD_T concentration in the fermentation liquor. Meantime, due to accumulation of straw, material output was difficult in the late period. Thus, on the 140^{th} d of the experiment, COD_T concentrations increased to 31.25, 37.98, 37.99, and 37.59 g/L, respectively. During this period, the proportions of COD_S to COD_T in the fermentation liquor in R1–4 reactors all decreased because the hydrolysis and acidification were inhibited by the relatively decreased water activity due to the excessive increase of TS concentration in the reactors of high temperature fermentation with the increase of loading. The proportions of COD_S to COD_T in the fermentation liquor all decreased and maintained at 21.33–36.54%. During the overload period, the ammonia nitrogen concentration increased dramatically. On the 140^{th} d of the overload period, the ammonia nitrogen concentration in the fermentation liquor in R1–3 and 7 reactors reached 4216.39 ± 325.32, 3566.28 ± 222.31, 3255.21 ± 156.32, and 1573.28 ± 99.98 mg/L, respectively. There were two reasons for the inhibition of dry fermentation in the whole overload period (Linke 2006; Pontes and Pinto 2006; Fan *et al.* 2008; Wang and Pang 2009; Lu *et al.* 2012; Feng and Li 2013; Luo and Li 2018; Zheng *et al.* 2018).

One reason was the acid inhibition. Too low pH in the anaerobic digestion process inhibited the activity of methanogens and decreased the rate of their enzymatic reaction, resulting in unstable gas production and operation of the system. The other reason was the ammonia nitrogen inhibition. In the anaerobic digestion system with longtime operation, with the increase of loading and TS concentrations, the ammonia nitrogen concentration in the digestion liquor increased up to the toxic concentration for the anaerobic digestive methanogens inhibiting their activity. Meantime, gas production and stability of the system began to decrease.

The TS concentration, biogas production rate, COD_T concentration, the proportion of COD_S to COD_T concentration, and the ammonia nitrogen concentration in R2 reactor increased with the increase of the loading frequency. HRT decreased in this stage, and the biological degradation rate decreased to 48.35%. There was balanced development of hydrolytic and acidogenic flora, H_2 and acetic acid producing flora, and specific anaerobic methanogens in this system in this stage. With the increase of the loading frequency, the continuous supply of organic matter to the reactors satisfied the requirements of hydrolytic and fermentation flora for nutrients. Organic matter in chicken manure and straw, such as starch and a small part of cellulose, was hydrolyzed to small organic acids and monosaccharides. Proteins were hydrolyzed to amino acids, which were transformed to organic acids and ammonia by deamination. Some of lipids were hydrolyzed to lower fatty acids and alcohols, such as acetic acid, propanoic acid, butanoic acid, long chain fatty acids, ethanol, CO_2, H_2, NH_3, and sulfides. Organic acids with more than three carbon atoms, long chain fatty acids, aromatic acids, and alcohols *etc*. were further decomposed to generate acetic acid and H_2 by bacteria producing H_2 and acetic acid as well as by bacteria reducing sulfides. However, pH of the system was maintained above 6.8 after adjustment, which enhanced the metabolic activity of methanogens. The produced small organic acids were transformed to methane and CO_2, preventing the decrease of pH in the system.

The overload period (20 d, from 121–140 d): 2 kg material was added every day from the 121^{st} d. HRT (121–140 d) was 10 d. The TS concentration, COD_T concentration, and the ammonia nitrogen concentration in R2 reactor increased with the increase of the loading frequency. However, the biogas production rate, the proportion of COD_S to COD_T concentration as well as pH decreased sharply. In the late period of the overload stage, system stirring and material output became difficult due to ground straw expansion in the material because of high temperature and water adsorption.

The gas production decreased in the system. Chicken manure and straw were discharged directly without biological and chemical degradation during material output. The biological degradation rate decreased to 22.35%. During the whole overload period, the hydrolysis and acidification were inhibited by the decreased water activity in the reactors caused by the excessive increase of TS concentration in the high temperature dry fermentation reactors because of the increased loading. Meantime, the easily degradable organic matter content was high. PH of the system decreased with small organic acids due to the metabolism of hydrolytic acidogenic microorganisms, which further inhibited the activity of methanogens and decreased the corresponding enzymatic reaction rate, leading to the unbalanced proportion of flora in the system as well as to unstable gas production and operation. Meantime, due to inhibition of the ammonia nitrogen, the ammonia nitrogen concentration increased to a level toxic for anaerobic digestive methanogens with the increase of the loading and TS concentration in the system with long term operation. The activity of methanogens was thus inhibited. Besides, stability and gas production ability of the system began to decrease.

Conclusion

(1) According to the changes of the loading frequency, the biogas production rate, pH, COD, and the ammonia nitrogen concentration in four reactors of high temperature dry fermentation of chicken manure and straw R1–3 and R7, the whole experimental process of high temperature dry fermentation of chicken manure and straw was divided into four stages, namely the stagnant adaptation period (20 d, from 1–20 d), the quick start period (30 d, from 21–50 d), the stable period (70 d, from 51–120 d), and the overload period (20 d, from 121–140 d). Concentration parameters of the system differentiated due to different loading

frequencies in different stages. During 70 days of the stable stage, material input and output were realized every day with up to 20% TS concentration in the system, stable gas production, and high biogas and methane production rates, realizing high temperature dry fermentation.

(2) The stagnant adaptation stage: during 20 days from the first loading (1–10 d), COD_T concentration in R1–4 of high temperature dry fermentation demonstrated a decreasing trend and decreased to 1.18, 1.34, 1.41, and 2.54 g/L on the 10^{th} d, respectively, and the proportions of COD_S to COD_T concentration decreased to 21.00, 22.04, 22.18, and 14.11%, respectively, whereas the ammonia nitrogen concentration increased to 594.21 ± 28.65, 528.39 ± 36.65, 511.07 ± 24.98, and 309.45 ± 33.65 mg/L, respectively.

(3) The quick start stage (30 d, from 21–50 d): COD_T concentrations in the digestion liquor in R1–4 reactors of high temperature dry fermentation increased with the increase of the loading frequency. The proportions of COD_T in R1–4 reactors on the 50^{th} d were 9.45, 11.21, 13.58, and 16.22 g/L, respectively. The proportions of COD_S to COD_T concentration in the fermentation liquor increased from 31.22, 33.45, 31.45, and 23.21% on the 21^{st} d to 43.25, 49.21, 44.35, and 30.05% on the 50^{th} d, respectively. Besides, the ammonia nitrogen concentrations increased from 822.56 ± 26.65, 798.74 ± 15.32, 756.38 ± 45.65, and 427.08 ± 25.32 mg/L on the 21^{st} d to 1100.19 ± 18.22, 872.38 ± 55.37, 838.28 ± 66.12, and 555.39 ± 88.77 mg/L on the 50^{th} d, respectively.

(4) The stable stage (70 d, from 51–120 d): COD_T concentrations in the digestion liquor in R1–4 reactors of high temperature dry fermentation increased from 13.45, 15.32, 16.11, and 18.11 g/L on the 51^{st} d to 17.89, 20.32, 22.65, and 25.87 g/L on the 120^{th} d, respectively, with the increase of the loading frequency. In the stable stage, the proportions of COD_S to COD_T in the fermentation liquor in R1–4 reactors increased and maintained at 45.65–47.21, 51.01–55.32, 44.12–48.82, and 32.01–39.15%, respectively. With the increase of the loading frequency, the ammonia nitrogen concentration increased to 2688.91 ± 68.35, 2479.45 ± 244.36, 2307.01 ± 175.36, and 1445.22 ± 88.32 mg/L on the 120^{th} d, respectively.

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