

Biodegradation Mechanism of Biogas Production by Modified Rice Straw Fermentation

Enhai Liu,^{a,*} Baozhong Zhu,^a Shengyong Liu,^b Hailong Yu,^a Zhiping Zhang,^b Huifan Zheng,^c JiaxinPan,^d and Wenyun Zhang^a

Based on the literature on the degradation mechanism and the change of micro-functional groups in the fermentation process of modified rice straw, this study aimed to solve the problems of low biogas production rate and poor stability of the biogas production system. In this work, mathematical equations were developed and combined with duck dung and rice straw mixed raw material to perform a fermentation test. The molecular micro-functional group changes of cellulose, hemicellulose, and lignin were studied to obtain the optimal ratio of mixed raw materials for fermentation and to explore the optimization mechanism of its fermentation biogas production. Experimental results showed that the optimal ratio of mixed raw materials was 2.8:1, and the inclusion of a suitable amount of Mn^{2+} (concentration of $2 \text{ mol} \times L^{-1}$) was able to strengthen MnP activity and improve the ability of white-rot fungi to rupture β -O-4 bonds. A modification pre-treatment *via* activated carbon-based solid acid was performed, and the experimental group generated 15.8% more cumulative biogas than the control group. The biogas yield reached its peak when 300 g of inoculum was added to the pre-treatment at a concentration of 30%.

Keywords: Biomass; Modification pre-treatment; Biogas; Degradation mechanism; Fermentation

Contact information: a: School of Petroleum Engineering, Changzhou University, Changzhou 213164, China; b: Key Laboratory of Renewable Energy of Ministry of Agriculture, Henan Agricultural University/Collaborative Innovation Center of Biomass Energy, Zhengzhou 450002, China; c: School of Energy & Environment, Zhongyuan University of Technology, Zhengzhou 450007, China; d: CSCEC Zhong Yuan Architecture Design Institute Co., Ltd., Zhengzhou, Henan Province 450042;

* Corresponding author: liuenhai1018@126.com

INTRODUCTION

Economic development imposes pressures on society with regard to energy shortages and environmental degradation. Fossil fuel scarcity and pollution are becoming increasingly serious. Consequently, the development and utilisation of bio-energy is increasingly essential, and it is of great theoretical and practical importance for the adjustment of China's energy structure, reforms in the production and consumption modes of energy, and environmental protection (Liu *et al.* 2006; Chu 2011; Mao *et al.* 2015; Li 2017). Biogas is a renewable bio-energy obtained from the anaerobic fermentation of crop stalks and animal breeding and from landfills containing municipal household waste (Møller *et al.* 2004; Svensson *et al.* 2005). The research and application of fermentation/biogas production technologies have been developing rapidly; many scholars have conducted in-depth studies of biogas production and fermentation technologies (Metha *et al.* 1990; Tuomela *et al.* 2000; Li *et al.* 2009; Song *et al.* 2012; Song 2013; Wang 2015; Yun 2015). In the 1930s, Buswell and Hatfield (1936) and then later Zhang *et al.* (2008) began their studies on the correlation between anaerobic microorganisms and gas

production conditions. In the 1940s, the technology of dry anaerobic fermentation in large quantities began to be used in Germany, France, and Algeria (Dan and Wei 2010). Lange and Ahring (2001) and Yu *et al.* (2008) named the methanogenic bacteria for the first time and separated them from methanotrophs that metabolise methane as their source of energy. At the beginning of 2009, the world's largest bio-genic gas production plant was put into operation in eastern Germany. Studies of biogas technology in China began in the 1930s, and the construction of relevant facilities started in the 1950s and 1960s (Li *et al.* 2008). Research into the utilisation of biogas digesters was developed (Wang *et al.* 2012; Chen *et al.* 2016). Statistics show that there were 12.9 million small biogas pools of 6 to 8 m² in China's rural areas by 2003 (Chen *et al.* 2010). The total number of completed biogas pits for household use exceeded 26.5 million by the end of 2007. At present, domestic experts and scholars have undertaken extensive research on biogas productivity and raw materials used for fermentation. Zhou *et al.* (2010) pre-treated straw *via* NaOH to study the influence of adding different amounts of NaOH and using different load rates on biogas production. Gao *et al.* (2010) studied the influence of rice straw compost pre-treatment on biogas production and estimated the loss of gas yield against total solid concentration caused by such pre-treatment. Yan *et al.* (2009) examined the effect of the addition of compound bacterium agent during the pre-treatment on gas production, and they concluded that corn stalk produced more biogas after pre-treatment with a compound bacterium agent than the control group, which had no such pre-treatment. Li *et al.* (2008) explored biogas productivity *via* using different straw types as the raw materials and pig dung as the inoculum. Zhou *et al.* (2010) conducted a comparative experiment, in which they compared the deconstruction rate and biogas yield of fresh straw, dry straw, and silage straw that were present in the same amount of dry matter at a medium temperature of 35 °C. The results showed that pre-treated silage straw effectively improved anaerobic digestion efficiency and gas production. Hu *et al.* (2010) pre-treated the straw with *Aspergillus niger*, *Penicillium*, *Rhizopus*, and their composite inoculants to test their influence on gas yield after such pre-treatment. Ai *et al.* (2010) worked on the process of pre-treatment for anaerobic straw fermentation and developed a quadratic polynomial mathematical model for biogas production. Li *et al.* (2007) focused on the changes in gas yield, acetic acid concentration in the fermented broth, and methane content during straw fermentation under medium, high, and ambient, temperatures.

Crop output in China's Central Plains Economic Zone increases every year, which creates a large amount of biomass resource, such as crop stalks (Zhang *et al.* 2006). At present, biogas production *via* fermentation is improving rapidly, and it has been applied to industrial production and daily life (Fan *et al.* 2008; Liu *et al.* 2013; Yang 2014). As the long, cold winter in the Central Plains Economic Zone results in low air temperature and ground temperature, low biogas productivity, poor system stability, insufficient gas supply, and inadequate utilisation constrain the application of biogas technologies to some extent. Based on available literature (Braber *et al.* 1995; Chen and Li 2002; Keller *et al.* 2003; Talebnia *et al.* 2010; Song *et al.* 2010; Guo *et al.* 2011; Chu *et al.* 2017), research to date has focused on enhancing gas yield by changing the fermentation environment and improving the anaerobic fermentation process, which has been applied to diverse straw fermentation sectors. However, systematic research into the mechanism of straw fermentation and biogas production is scarce, which inhibits the development of biogas technologies and its promotion.

This research included several fermentation experiments on a mixture of duck dung and rice straw as the raw material. 1. Given the *de facto* biogas production condition, a

modification pre-treatment *via* activated carbon-based solid acid was completed before the commencement of fermentation to increase the rate of hydrolysis during the rice straw fermentation process. The theoretical analysis of cellulose crystallinity changes revealed the degradation mechanisms of cellulose, hemicellulose, and lignin in rice straw during fermentation. 2. Through mathematical equation description and analysis, a degradation kinetics model was established, and it fit the curve equation of organic acid concentration over time during the fermentation process of mixed raw materials. Further, the addition of Mg^{2+} , Mn^{2+} , Cu^{2+} , and other metal ions to the inoculum fermentation broth was investigated, and the changes in the molecular microfunctional groups of cellulose, hemicellulose, and lignin were explored, which revealed the changing characteristics of the added catalysts to optimize the degradation of microfunctional groups by anaerobic fermentation and the synergistic mechanism of various parameters during the fermentation of mixed raw materials. 3. The added catalyst was characterized and analyzed, and the mechanisms of white-rot fungi's lignin enzymatic hydrolysis and lignin benzene ring structural degradation were explored. In addition, the efficiency and temperature adaptability of biomass degradation and micro-functional group change technology during the fermentation of mixed raw materials for biogas production was revealed. The difficult-to-degrade components in rice straw were discussed technically, and the molecular structures of the more difficult-to-degrade components in the fermentation process of rice straw were examined. Such components were then modified accordingly to explore the best process conditions for analyzing the fermentation of mixed raw materials. The theoretical basis for the enzymatic hydrolysis of lignin and the degradation of the lignin benzene ring structure by the white-rot fungi were enriched, and the methods to increase the amount of catalyst and the temperature range of anaerobic fermentation were broadened.

EXPERIMENTAL

Materials

Rice straw that was available in the science and educational experiment farm located in the Central Plains Economic Zone (Xinyang, China) (fresh straw with roots removed, naturally air dried, chopped into 2 to 3 cm pieces, and soaked in a biogas slurry with water before storage) and fresh duck dung collected from the Cherry Valley duck breeding and processing bases (Xinyang, China) located in the Central Plains Economic Zone were selected as raw materials for this fermentation research. The components of rice straw determined *via* sample analysis are shown in Table 1.

Table 1. Composition of Rice Straw

Volatiles	Fixed Carbon	Moisture Content	Ash Content	Cellulose	Hemicellulose	Lignin
68.81%	13.42%	5.41%	10.80%	42.30%	18.45%	16.17%

Test design (Liu 2015; Liu et al. 2017)

The control group consisted of smashed straw that was transferred to a 10-L fermentation tank equipped with a JJ-IA electric mixer (Experimental Instrument Company of Jintan District, Jiangsu Province, China) (speed: 3000 rpm), and a suitable amount of

water was added for a 6-day fermentation trial (the mixture was stirred when necessary) before storage for later use.

The experimental group consisted of crushed straw that was transferred to a 10-L fermentation tank equipped with a JJ-IA electric mixer (speed: 3000 rpm), and 50 g of activated carbon-based solid acid (obtained from rice straw by the phosphoric acid method and washed with 200 mL of deionized water) prepared in advance at the laboratory was added for a 6-day pre-treatment (a small amount of CO₂ gas was generated in the fermentation process that was not counted in the total biogas yield) before storage for later use. The control group and the treatment group (35°C, biogas slurry concentration 8%) were prepared as shown in Table 2.

Table 2. Control Group and Treatment Group (35 °C and 8% Biogas Slurry Concentration)

Group	Test Design	Inoculum Concentration (%)	Inoculum Added (g)
Control group	U1	20	200
	U2	30	300
	U3	40	400
Experimental group	R1	20	200
	R2	30	300
	R3	40	400

Test facilities

In this research, a self-made fermentation apparatus was used to collect gas *via* the drainage method. The apparatus consisted of a 2.5-L fermentation tank for fermentation, a 2.5-L air collector to collect gas *via* the drainage method, and a water collector to collect water. The fermentation tank was equipped with a JJ-IA electric mixer (speed: 3000 rpm) and a PC-1000 digital temperature controller (Experimental Instrument Company of Jintan District, Jiangsu Province, China), in the bottom of which was a gas sampling port. The daily gas yield was recorded to track the changes in gas production rate and accumulative yield in the control and experimental groups, which had the same fermented broth concentration, different inoculum concentrations, and different fermentation temperatures (Fig. 1).

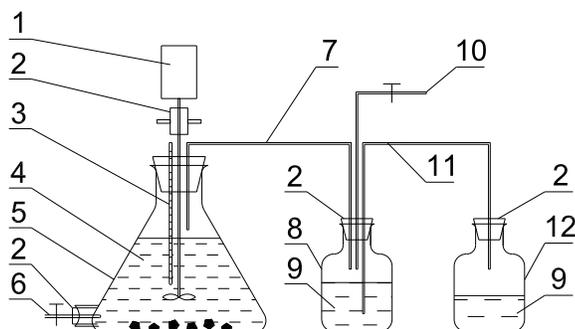


Fig. 1. The apparatus for this fermentation experiment (Liu *et al.* 2017), which included (1) the mixer, (2) rubber stopper, (3) temperature controller, (4) fermented broth, (5) fermentation tank, (6) sampling opening, (7) airway tube, (8) gas collector, (9) water, (10) gas collection opening, (11) aqueduct, and (12) water collector

Mechanism of Biomass Degradation during Fermentation

Rice straw is mainly composed of cellulose, hemicellulose, and lignin, which interweave to comprise the cytoderm of rice straw. Cellulose is a linear polymer formed in the cytoderm from glucose molecules connected by ether bonds. Hemicellulose mainly consists of xylose and mannose. Lignin has a complex 3-d structure that originates from the disordered polymerisation of benzene propane monomer substituted by alkyl or methoxy. Cellulose in rice straw is the main source of biogas. However, the strong intermolecular hydrogen bond results in an orderly crystal structure, which, together with the hydrophobic surface, determines the ease with which crystalline cellulose is hydrolysed by enzymes or acids. Therefore, during the rice straw fermentation process, changes in gas production can be measured by variation in the crystallinity of cellulose (Song *et al.* 2012; Liu 2015).

Variations in the degree of crystallinity of cellulose

X-ray diffraction (XRD) technology was used to analyse the dynamic variation of the crystallinity and crystallisation size within straw particles. With a D/Max-2400 powder X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) (test conditions: Cu target radioactive source, X light wavelength of $\lambda=0.15406$ nm, tube voltage of 40 kV, tube current of 60 mA, scanning speed of $8^\circ/\text{min}$, step length of 0.02° , scanning angle 6° to 70°), the diffraction pattern and the crystallinity within the straw particles was calculated with Eq. 1. The crystallinity of cellulose for control group I and treatment group II at different times is shown in Fig. 2. The crystallinity (%) is given by Eq. 1,

$$C_r(\%) = (I_{002} - I_{am}) / I_{am} \times 100 \quad (1)$$

where I_{002} is the maximum intensity of the diffraction angle of (002) lattice (the diffraction intensity of the crystalline region), and I_{am} is the scattering intensity of the diffraction at the amorphous area, at $2\theta=18^\circ$.

During the test, three sets of data were tested and recorded, and the average value was taken. Figure 2 shows that the crystallinity of cellulose in the control group was approximately 35% when fermentation commenced. At the beginning of fermentation, cellulose, hemicellulose, and lignin were gradually enzymolysed. Non-crystalline components, such as hemicellulose, lignin, *etc.*, began their enzymolysis first, followed by components in the order of decreasing crystallinity of cellulose. Crystallinity decreased to a minimum level on the 20th day, which generated the highest daily gas yield. During the later period of the enzymolysis/fermentation process, the proportion of crystallisation increased and gradually stabilized. Throughout the fermentation process, the crystallinity of cellulose in Material II from the experimental group was always lower than that in Material I from the control group, which was attributed to the strong acid site of the activated carbon-based solid acid. The proton in the sulfonic acid group undermined the glucosidic bonds in the cellulose. This prevented the combination of vinegar bonds and hydrogen bonds between lignin and polysaccharides, and it enhanced the separation and deconstruction of cellulose, hemicellulose, and lignin. This facilitated bacterial enzymolysis in the biogas slurry and increased the gas yield from the fermentation process. Compared to the fermentation/gas production process at a biogas slurry concentration of 20%, gas yield reached a peak with the lowest crystallinity of cellulose when concentration rose to 30%. At this point, the large amount of bacteria in the slurry boosted the enzymolysis/fermentation of cellulose, hemicellulose, and lignin in the rice straw. However, it was not always productive at higher concentrations, and excessively high

concentrations affected the activity of bacteria, which resulted in a decline in enzymolysis efficiency.

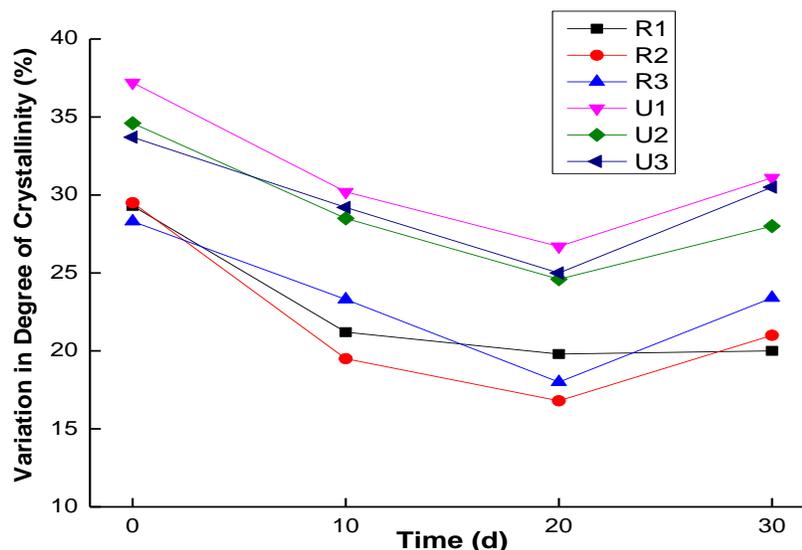


Fig. 2. Variations in the degree of crystallinity of cellulose

Variation of Acid Concentration

Measurement of organic acid content

During the test, three groups (labeled as A, B, and C) of the same test materials were tested, and the average of the three groups of data were taken as the results. The raw materials for this fermentation test were fresh duck dung and prepared straw, which were mixed in ratios of 1:1, 1.8:1, 2.8:1, and 3.8:1 by dry matter. The inoculum was the biogas slurry collected from the last fermentation trial.

To perform the experiment, fresh duck dung and prepared rice straw was poured into the glass and sealed for stack retting for 4 d to 6 d before being transferred to the fermentation tank. The biogas produced was collected by the gas collector (Liu *et al.* 2017).

Gas chromatography-mass spectrometry (G3440B) (Agilent 7890B-GC; Agilent Technologies Inc. Beijing, China) was employed to measure the content of organic acids (acetic acid, propionic acid, and butyric acid) in the experimental group II during fermentation. The variation of the concentration of acetic acid, propionic acid, and butyric acid over time is shown in Figs. 3, 4, and 5. According to Figs. 3 and 5, the concentration of acetic acid and butyric acid increased rapidly in the early stage of fermentation and reached a peak on the 21st day. This was because the early stage of fermentation is primarily an acid producing phase, which means that the fermented bacteria absorb the small molecular compounds produced in the hydrolysis stage into their cells and enzymolyse them into volatile organic acids such as acetic acid, propionic acid, and butyric acid.

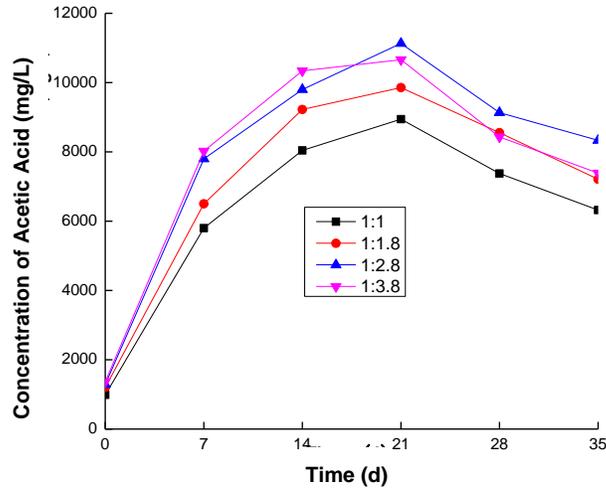


Fig. 3. The concentration of acetic acid over time

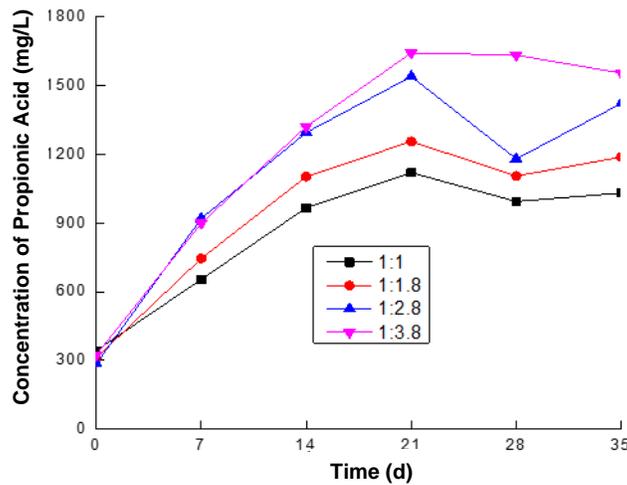


Fig. 4. The concentration of propionic acid over time

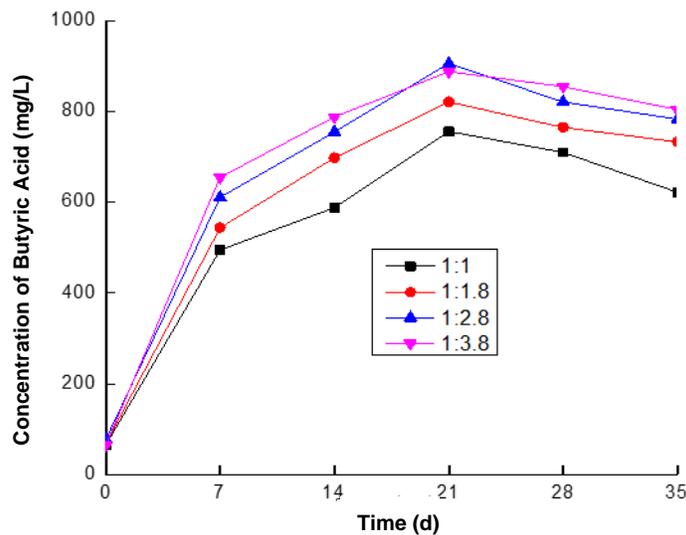


Fig. 5. The concentration of butyric acid over time

As the fermentation proceeds, the speed at which methanogenic bacteria decompose acetic acid into methane escalates, which results in a decrease in acid concentration. The fermentation tests at different ratios of raw materials showed that the concentration of acetic acid and butyric acid during the fermentation process gradually increased as the proportion of duck dung increased. The organic matter in duck dung is conducive to generating acetic acid, and hydrogen-producing acetogenic bacteria in duck dung can enhance the decomposition of rice straw. Nevertheless, the concentration of acetic acid and butyric acid during the fermentation process (when mixed in a ratio of 3.8:1) was lower than that at a ratio of 2.8:1 in the experimental group. This may have been due to the excessively high concentration of organic matter that inhibited the rate of acetic acid and butyric acid generation.

Figure 4 shows that the concentration of propionic acid increased rapidly in the early stage of fermentation, reached its peak on the 21st day, and then gradually stabilized. Further, the concentration of propionic acid increased as the ratio of raw materials by dry matter increased. According to Figs. 3, 4, and 5, the concentration of propionic acid reached its peak in the experimental group with a 3.8:1 ratio of raw materials, but the gas yield was lower than that of the group with a 2.8:1 ratio of raw materials. This was because excessively high concentrations of propionic acid in the fermentation process restrained the activity of methanogens, which led to decreased methane production.

The fitted curve equation

In summary, the experimental group with a 2.8:1 ratio of raw materials generated the optimal results for the rates of acid production and methane production in the fermentation process. According to Figs. 3, 4, and 5, the fitting curve that illustrates the variations of the organic acid concentration over time is shown in Table 3.

Table 3. Fitting Curve of the Variations of the Organic Acid Concentration over Time

Acid	Equation of the Variations over Time (Y- Acid Concentration(mg/L); X- Fermentation Time (d))	Correlation Coefficient
Acetic acid	$Y = -19.16X^2 + 836.03X + 1886.94$	$R^2 = 0.91279$
Propionic acid	$Y = 0.08X^3 - 5.88X^2 + 142.23X + 255.61$	$R^2 = 0.85024$
Butyric Acid	$Y = -1.38X^2 + 65.76X + 126.14$	$R^2 = 0.93509$

Degradation kinetics during the fermentation process

Straw fermentation is a biomass degradation process in the anoxic environment that generates biogas. The degradation of rice stalk can be regarded as the superposition of the separate degradation processes of three components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose mainly produce organic acids *via* degradation to produce volatile gases, whereas lignin is mainly deconstructed into carbon. This test was conducted under a medium temperature (35 °C), and the kinetic equation of isothermal homogeneous reactions was adopted for analysis.

The activation energy and pre-exponential factors

The kinetic equation on biomass degradation is defined as $A_s + B_s = C_s$: A_s is the raw materials, B_s is the solid residue after degradation, and C_s is the gases generated from the

degradation process, which can be calculated according to Eq. 2 (Liu *et al.* 2013; Yang 2014),

$$\frac{d\alpha}{dt} = K(1-C)^n \quad (2)$$

where α represents the homogeneous reaction process, so $\alpha = [(W_0 - W)/(W_0 - W_\infty)]$, W represents the amount (kg) of biomass samples at time t , W_0 and W_∞ are the initial amount of the sample (kg) and the final residue (kg), respectively. In addition, $(1-C)^n$ represents the reaction order of the homogeneous reaction mechanism. Consequently, the kinetic equation of the isothermal homogeneous reaction for this degradation process was,

$$\frac{d\alpha}{dt} = A \exp\left(-\frac{E}{RT}\right)(1-C)^n \quad (3)$$

where E and A are the activation energy ($\text{kJ} \times (\text{mol})^{-1}$) and the pre-exponential factor in the degradation reaction, respectively, and R is the ideal gas constant ($8.314 \text{ J} \times (\text{K} \cdot \text{mol})^{-1}/\text{mol}$). Equation 4 gives the activation energy and pre-exponential factors (Wang *et al.* 2006; Liu *et al.* 2011; Liu *et al.* 2013; Yang 2014):

$$\frac{d\alpha}{dt} = \frac{A}{\beta} \exp\left(-\frac{E}{RT}\right)(1-C)^n \quad (4)$$

According to Eq. 4, when calculating, if $n=1$, Eq. 5 was used:

$$n=1, \quad \ln \frac{-\ln(1-\alpha)}{T^2} = -\frac{E}{RT} \ln \left[\frac{AR}{\beta E} \left(1 - \frac{2RT}{E}\right) \right] \quad (5)$$

According to Eq. 4, when calculating, if $n \neq 1$, Eq. 6 was used:

$$n \neq 1, \quad \ln \frac{1-(1-\alpha)^{1-n}}{T^2(1-n)} = -\frac{E}{RT} \ln \left[\frac{AR}{\beta E} \left(1 - \frac{2RT}{E}\right) \right] \quad (6)$$

For the general reaction zone and most of the activation energy values:

$$\frac{E}{RT} \gg 1, \quad 1 - \frac{2RE}{T} \approx 1 \quad (7)$$

When $n=1$, the curve was developed based on:

$$\ln \frac{-\ln(1-\alpha)}{T^2}, \quad \frac{1}{T} \quad (8)$$

When $n \neq 1$, the curve was developed, based on:

$$\ln \frac{1-(1-\alpha)^{1-n}}{T^2(1-n)}, \quad \frac{1}{T} \quad (9)$$

The activation energy E and the pre-exponential factor A was deduced from the slope $-E/R$ and intercept $\ln[(AR/(\beta E))(1-2RT/E)]$ of the straight line (Liu *et al.* 2013).

Measurement of Cellulose, Hemicellulose, and Lignin during Fermentation

Theoretical calculation (Fan et al. 2008)

Approximately $0.2 \text{ mol} \times \text{L}^{-1}$ sodium thiosulfate titration was adopted to measure the variations of the cellulose content in the fermentation process. The variation was calculated with Eq. 10:

$$N = \frac{C(V_a - V_b)}{m \times 24} \quad (10)$$

Oxidoreduction titration was adopted to measure the variations of the lignin content in the rice straw fermentation process. The variations were calculated according to the with Eq. 11,

$$N = \frac{C(V_a - V_b)}{m \times 48} \quad (11)$$

where C is the concentration of sodium thiosulfate ($\text{mol} \times \text{L}^{-1}$), V_a is the volume of sodium thiosulfate consumed by blank titration (mL), V_b is the volume of sodium thiosulfate consumed by the solution (mL), and m is the mass of rice straw (g).

Measurement of cellulose, hemicellulose, and lignin

A 30-day fermentation test (extended for 5 d) was conducted, and the mixture was stirred when necessary. During the test, three sets of data were tested and recorded, and the average value was taken. The variation in cellulose content over time during the fermentation process with the mixed raw material ratios of 1:1, 1.8:1, 2.8:1, and 3.8:1 by dry matter is shown in Fig. 6. An alkaline solution was adopted to measure the variation of the hemicellulose content in the rice straw fermentation process, as shown in Fig. 7.

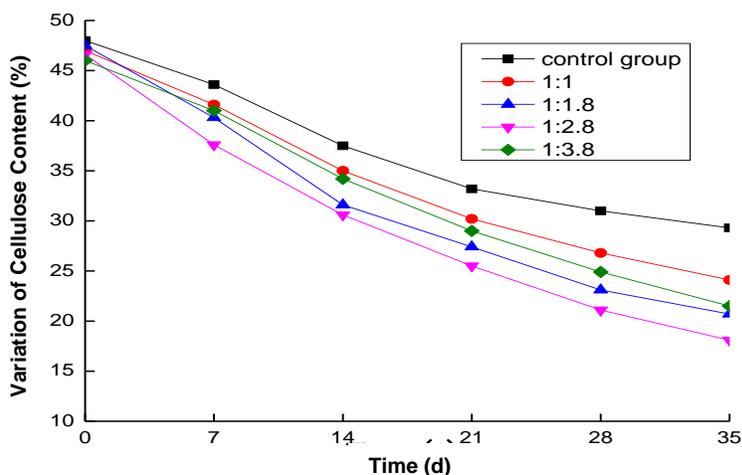


Fig. 6. The variation of cellulose content over time

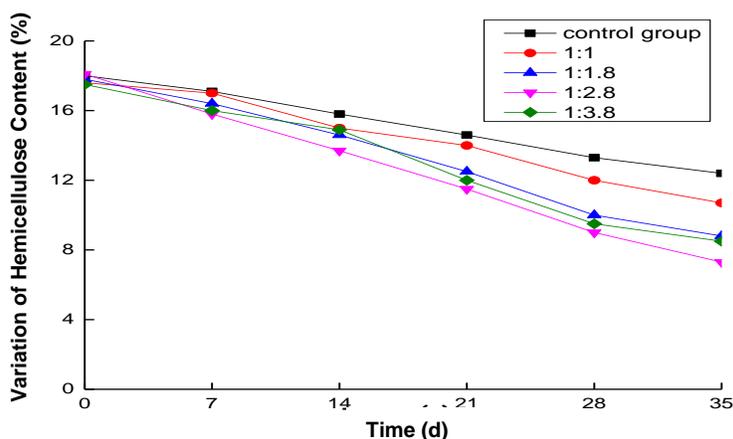


Fig. 7. The variation of hemicellulose content over time

The variation of lignin content over time during the fermentation process of a mixed raw material at differing ratios is shown in Fig. 8.

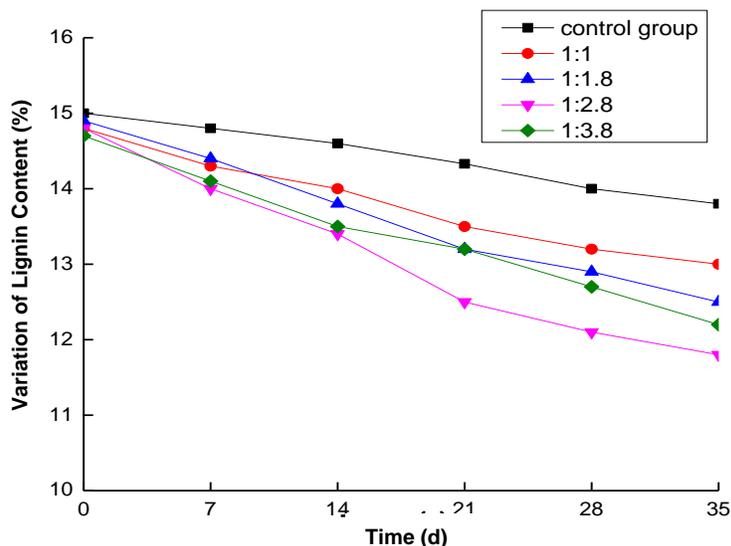


Fig. 8. The variation of lignin content over time

The fitted curve equation

As shown in Figs. 6, 7, and 8, the degradation rates of cellulose, hemicellulose, and lignin differed in the fermentation process depending on the different mixed raw materials ratios after pre-treatment with activated carbon-based solid acid. Cellulose, hemicellulose, and lignin degraded slowly in the control group without duck dung in the raw materials. The contents of cellulose, hemicellulose, and lignin decreased from 47.8%, 18%, and 15.1% at the beginning of fermentation to 29.3%, 12.4%, and 13.8%, respectively, upon completion of fermentation. In the fermented broth from the experimental groups that had raw materials mixed at ratios of 1:1, 1.8:1, 2.8:1, and 3.8:1, the contents of cellulose, hemicellulose, and lignin declined from the initial 47%, 18%, and 15%, to 24.1%, 10.7%, and 13%, respectively (with a 1:1 ratio of raw materials); 20.7%, 8.8%, and 12.5% (with a 1.8:1 ratio of raw materials); 18.1%, 7.3%, and 11.8% (with a 2.8:1 ratio of raw materials); and 21.5%, 8.5%, and 12.2% (with a 3.8:1 ratio of raw materials), respectively, upon completion of fermentation.

The results showed that added duck dung promoted the degradation/biogas production of cellulose, hemicellulose, and lignin in rice straw. This occurred because more strains were created from the duck dung, which was conducive to the degradation of cellulose, hemicellulose, and lignin. During fermentation, adding more duck dung was not always a good option, as excessive amounts of duck dung inhibited the activity of white-rot fungi, which reduced the rate of degradation of cellulose, hemicellulose, and lignin. Lignin was degraded in the same way as cellulose and hemicellulose but at a slower pace. The reasons for this were related to the following: first, lignin's more complex structure, which is a complex 3-d structure formulated after disordered polymerisation of benzene propane monomer substituted by alkyl or methoxy; second, the higher stability and lower degradation rate of lignin, as it has to provide sufficient strength and hardness for plant cells to avoid biological invasion and water erosion.

According to Figs. 6, 7, and 8, the fitting equations on the variation of the content of cellulose, hemicellulose, and lignin in the experimental group with a mixed raw material

ratio of 1:2.8 were developed (Fan *et al.* 2008; Song 2013; Wang 2015; Liu 2015), respectively, as:

$$Y = -0.81X + 43.99, \quad R^2 = 0.95708 \quad (12)$$

$$Y = -0.31X + 18.04, \quad R^2 = 0.99805 \quad (13)$$

$$Y = -0.09X + 14.64, \quad R^2 = 0.96805 \quad (14)$$

The conversion rate α was set to 0.8 by definition, where Y is defined as $\ln \beta$, and $\beta = \ln \frac{-\ln(1-\alpha)}{T^2}$; X is defined as T^{-1} . The curve was developed based on $\ln \beta$, T^{-1} . The curve was developed based on $\ln \beta$, T^{-1} , to work out the degradation kinetics parameters for the cellulose, hemicellulose, and lignin within rice straw, as shown in Fig. 9.

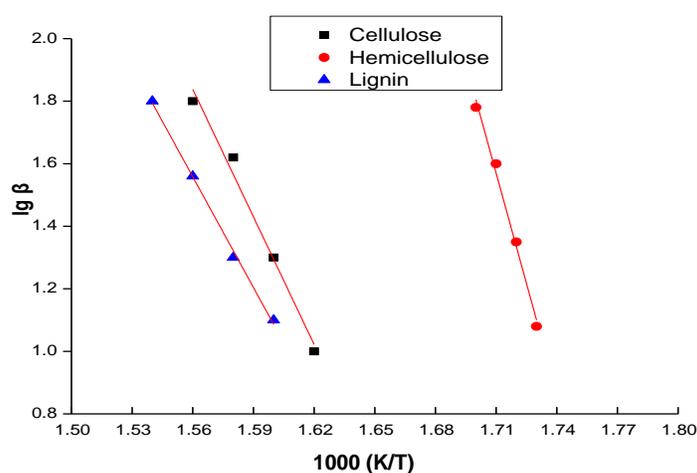


Fig. 9. The degradation kinetics parameters for cellulose, hemicellulose, and lignin

As shown in Fig. 9, a least squares fit was adopted to develop the curve, so as to deduce the slope and intercept for each group. The activation energies of cellulose, hemicellulose, and lignin in the degradation reaction were 192, 208, and 287 $\text{kJ} \times \text{mol}^{-1}$, and the pre-exponential factors were 5.23×10^{19} , 2.23×10^{34} , and 4.63×10^{18} , respectively. The three components were ranked in descending order of activation energy: lignin, hemicellulose, and cellulose. Therefore, lignin is a component of straw biomass that is more difficult to degrade.

The degradation of lignin (Feng et al. 2010; Song 2013; Yang 2014; Wang 2015)

Lignin in rice straw is mainly composed of phenylpropanoid polymer, which is made of guaiacyl, syringyl propane, *etc.* Its complex molecular structure involves diverse functional groups, such as hydroxyl, methoxy, carbonyl, and benzene ring structures. The benzene ring structure, which is the frame of lignin, is formulated from chemical bonds such as β -O-4, 4-O-5, β -5, 5-5, *etc.* This cyclic structure and the diversified chemical bonds determine the complexity when breaking down these bonds, and the difficulty of breaking these functional groups. This gives rise to lignin's amorphous nature, irregularity, and 3D network-like stability, which make lignin a stubborn part of the cytoderm plant. The 3D network structure of lignin can be regarded as a porous medium. When cellulases approach the internal cellulose surface through holes in the lignin, the complex structure blocks its path; cellulase is easily absorbed by the functional groups and fails to pass through these

holes, which results in lower cellulase activity. Then, the enzymolysis of cellulose is affected, which restrains the entire fermentation process.

The influence of white-rot fungi on lignin degradation

White rot fungi entail special mechanisms of extracellular enzymolysis and free radical degradation, which can effectively enhance the enzymolysis process, thus increasing the rate of passage of cellulases through the holes in lignin. With the focus on the enzymolysis mechanism of white-rot fungi on lignin, a modification test was conducted to address the difficulty of degradation of benzene rings in lignin. This study of the degradation of the benzene ring was performed by adding Mg^{2+} , Mn^{2+} , Cu^{2+} , and other metal ions to the broth.

During the test, three sets of data were tested and recorded, and the average value was taken and edited and processed using Excel.

1. The experimental steps were as follows: 1 kg of prepared rice bar was placed in the 10-L fermentation tank, dosed with 500 mL of NaOH solution (6% mass fraction), 200 g of solid culture containing white-rot fungi (prepared in the biomass laboratory) and a suitable amount of water for the fermentation test (the mixture was stirred when necessary). The experiment was conducted using *Phanerochaete chrysosporium* as a strain and using potato, wheat bran, sugar, and other raw materials in a constant temperature incubator at 26 °C. After this pre-treatment, 1 kg of mixed fermented broth was poured into the 2.5-L fermentation tank (biogas slurry with duck dung used as the inoculum), dosed with 10 g of $MgSO_4$, 10 g of $MnSO_4$, and 10 g of $CuSO_4$ for each group. Fermentation tests were carried out at 35 °C to explore the enzymolysis effect of white-rot fungi on lignin and the influence of metal ions on the degradation of functional groups in lignin. The variations in lignin content in the fermentation process from each group are illustrated in Fig. 10.

2. The control group: no white-rot fungi or compounds were added during pre-treatment.

3. The benzene structure is difficult to degrade. To analyse the contribution of Mg^{2+} , Mn^{2+} , and Cu^{2+} to the degradation of this ring structure, the gas chromatography-mass spectrometry (G3440B) (Agilent 7890B-GC; Agilent Technologies Inc., Beijing, China) and biogas analyzer (RJM/B-ZQ) (Rijie Instrumentation Equipment Co., Ltd. Beijing, China) were adopted to measure the samples during fermentation to study the variations of the peaks of the benzene ring structure, as shown in Fig. 11.

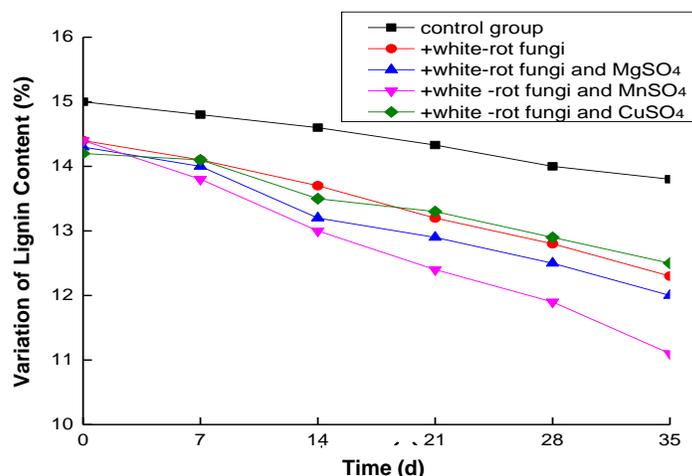


Fig. 10. The variation of the lignin content in the fermentation in each group

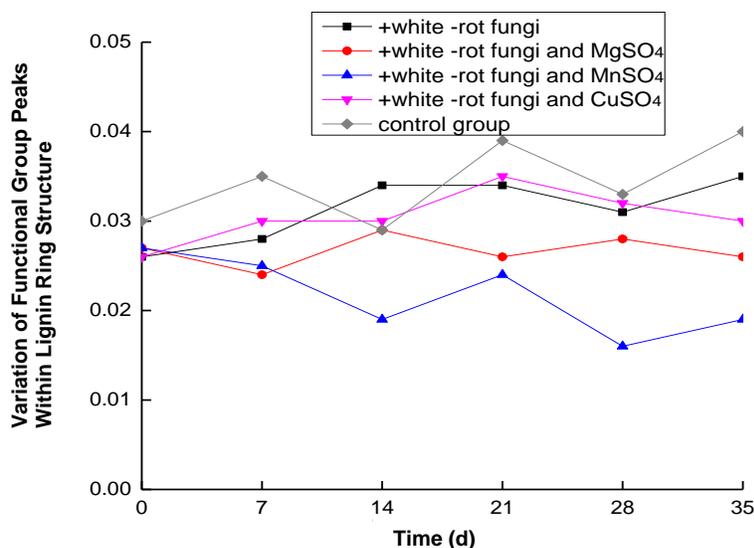


Fig. 11. The variation of the peaks of functional groups within lignin's ring structure in each group

Figures 10 and 11 show that in the control group where no white-rot fungi were added to the pre-treatment process, the lignin content decreased from 15% to 13.8% during the fermentation test. In contrast, in the experimental groups, small-scale degradation started during pre-treatment when white-rot fungus was added. During fermentation, the lignin content decreased from 14.4% to 12.3%, which indicated that white-rot fungi were more effective at deconstructing lignin, or rather its extracellular enzymolysis had a stronger degradability. The addition of MgSO₄ and MnSO₄ during fermentation caused the lignin content to decrease to 12% and 11.1%, respectively, and the lignin content decreased to 12.5% with the addition of CuSO₄ solution, which was approximately the same level as that of the control group pre-treated with only white-rot fungi. These results suggested that different metal ions exerted varying influences on the activity of white-rot fungi. Mg²⁺ and Mn²⁺ increased the activity of white-rot fungi, whereas Cu²⁺ had little effect or may have constrained such activity. Therefore, Mn²⁺ was the most effective at boosting the degradation of the benzene ring structure if added during the fermentation process, followed by the Mg²⁺ ion. The addition of Cu²⁺ had no obvious effect.

Influence of Mn²⁺ Concentration on MnP Activity and Lignin Functional Groups

The variation of MnP activity with concentration of Mn²⁺

The prepared straw was soaked in the acetic acid-sodium acetate buffer solution for 4 h. Then, 0.5 mol × L⁻¹, 5 mol × L⁻¹, and 10 mol × L⁻¹ of MnSO₄ solution and 0.5 mol × L⁻¹ of H₂O₂ solution were prepared. Upon the addition of 2 mL of each solution, the variation in MnP activity was recorded by measuring and recording the absorbance value at 270 nm. MnP was an enzyme that can degrade lignin (phenol-containing lignin complex), which could oxidize dimer phenol. The addition of Mn²⁺ ions during the fermentation of rice stalks could increase the enzymatic activity of MnP, enhance the ability to destroy the benzene ring structure of lignin, and promote fermentation gas production. The influence of different Mn²⁺ concentrations on MnP activity is shown in Fig. 12.

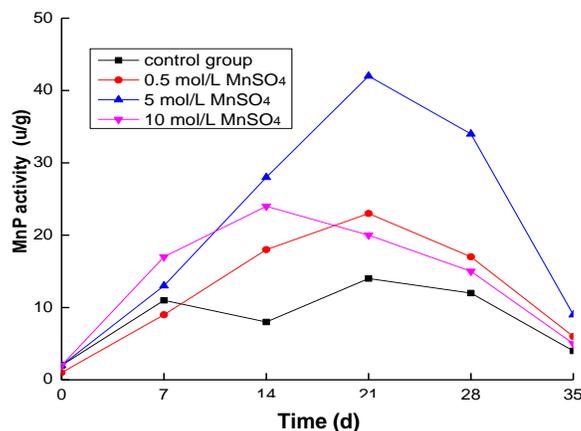


Fig. 12. The influence of different Mn^{2+} concentration on MnP activity

Figure 12 shows that the activity of extracellular MnP increased gradually in the early stage of fermentation and reached a peak of $14 \mu\text{g}^{-1}$ on about the 21st day. Thereafter, the activity decreased. As for the experimental groups dosed with $0.5 \text{ mol}\times\text{L}^{-1}$, $5 \text{ mol}\times\text{L}^{-1}$, and $10 \text{ mol}\times\text{L}^{-1}$ of $MnSO_4$ solution, the extracellular MnP activity was increased in a similar manner to that in the control group during the first week of fermentation. However, the increase accelerated in the later stages of fermentation. Maximum rates of 2314, 4214, and 2414 μg^{-1} were recorded for the three groups. Extracellular MnP activity was higher than that in the control group. The increased MnP activity in the 0.5 and $5 \text{ mol}\times\text{L}^{-1}$ experimental group was higher than in the control group. All of them reached a peak in the third week; however, the MnP activity in the $10 \text{ mol}\times\text{L}^{-1}$ experimental group reached its peak in the second week and then gradually decreased. This may have been related to the excessively high Mn^{2+} concentration. The MnP activity in the $5 \text{ mol}\times\text{L}^{-1}$ experimental group was stronger. With the added $MnSO_4$ solution, the Mn^{2+} concentration was increased to $2 \text{ mol}\times\text{L}^{-1}$ in the fermented broth. This resulted in enhanced MnP activity, which strengthens its ability to destroy the benzene ring structure of the lignin to promote the degradation of lignin and improve fermentation/biogas production efficiency.

The influence of Mn^{2+} concentration on the functional groups in lignin

The fermented broth pre-treated with white-rot fungus was dosed with $MnSO_4$ solution to increase the concentration of Mn^{2+} to approximately $2 \text{ mol}\times\text{L}^{-1}$. Then, the infrared spectrum was adopted to analyse and measure the variation of guaiacyl and syringyl propane in lignin. The comparative results from the test assessing the influence on the benzene ring structure *via* modification after pre-treatment and 28-day fermentation are presented in Fig. 13.

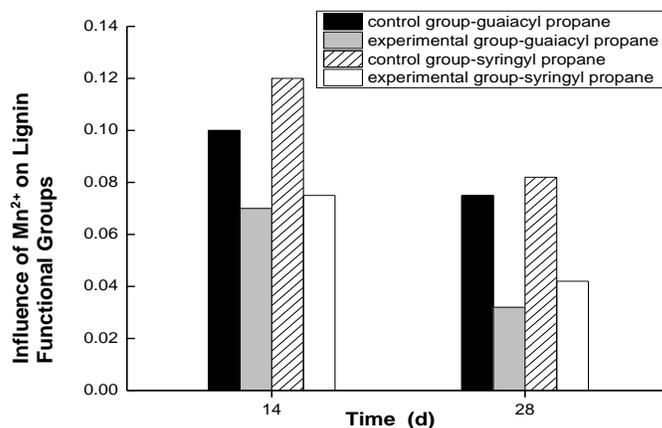


Fig. 13. The influence of Mn²⁺ on lignin's functional groups

Figure 13 shows that, compared to the control group, the guaiacyl and syringyl propane in the experimental group was effectively deconstructed after pre-treatment. After a two-week fermentation trial, the peaks of the guaiacyl and syringyl propane in the experimental group decreased approximately 30% and 37.5%, respectively. After four weeks, the guaiacyl and syringyl groups decreased approximately 57% and 48.7%, respectively. These results showed that Mn²⁺ enhanced the ability of white-rot fungi to destroy the β -O-4 bond and thus enhance MnP activity to improve the degradation and modified properties of lignin.

Fermentation and Gas Production of Modified Rice Straw

Gas yield is an important indicator of the performance of biogas fermentation processes. In response to the *de facto* production conditions for fermentation/biogas production *via* the duck dung and straw mixture, a modification pre-treatment *via* activated carbon-based solid acid (6 d) was conducted before rice straw fermentation.

Test method (Svensson et al. 2005; Liu 2015; Liu et al. 2017)

Rice straw, after pre-treatment *via* activated carbon-based solid acid, was added to biogas slurry containing 8% total solids prepared with fresh water for both the control and experimental groups. Then, for both groups, 1000 g of fermented broth was added into the fermentation tank with the inoculum, which was the biogas slurry from the last fermentation (a mixture of fresh duck dung and prepared straw in a 2.8:1 ratio by dry matter with a moisture content of 84.1%). The fermentation was designed to run under a medium temperature (35 °C), and three groups of the same experimental materials (labeled A, B, and C) were used. The fermentation time was set to 30 d (from March 4, 2017 to April 2, 2017), and the mixture was stirred when necessary. The change in gas production rate in the experimental/control group (35 °C and 8% biogas slurry concentration) is listed in Table 4.

Table 4. Changes in Gas Production Rate in the Experimental and Control Groups (35 °C and 8% Biogas Slurry Concentration)

Test Group		Inoculum Concentration (%)	Inoculum Added (g)	A Group Cumulative Gas Yield (mL)	B Group Cumulative Gas Yield (mL)	C Group Cumulative Gas Yield (mL)	Cumulative Average Gas Production (mL)
Control group	U1	20	200	14832	14831	14835	14833
	U2	30	300	22598	22607	22604	22603
	U3	40	400	21492	21494	21498	21495
Experimental group	R1	20	200	23947	23949	23948	23948
	R2	30	300	26170	26176	26172	26173
	R3	40	400	25297	25301	25300	25299

Result analysis

Table 4 shows that, under the same fermentation temperature (35 °C) and the same biogas slurry concentration (8%), the inoculum concentration and the amount of inoculum added exerted a noticeable influence on the gas yield, as shown by the anaerobic fermentation processes in both control group I and experimental group II. The average gas yield for the groups labeled A, B, and C reached a peak when the batch was dosed with 300 g of inoculum at a concentration of 30% (22,603 mL in the control group and 26,173 mL in the experimental group). When the inoculum concentration was changed between 20% and 30% and the added inoculum dose varied between 200 g to 300 g, the gas yield increased as the biogas slurry concentration and the added inoculum dose increased, as robust microbial metabolic activity leads to high production rates and a larger yield. When the inoculum concentration ranged between 30% and 40% and the added inoculum mass was between 300 g and 400 g, the gas yield decreased as the biogas slurry concentration and added inoculum dose increased. At this point, the influence of inoculum concentration and the added amount on the gas yield was reduced, as excessively high concentrations constrain microbial metabolic activity. In addition, fatty acid concentrations increased in the fermented liquid, which led to its acidification. As a result, the gas yield declined (Liu *et al.* 2017).

The results showed that, after pre-treatment by activated carbon-based solid acid (6 d), the cumulative gas production of the experimental group II increased 15.79% more than that in the control group. The yield reached a peak when the inoculum concentration was 30% and the added volume was 300 g.

CONCLUSIONS

1. Based on the analysis on the degradation mechanism of cellulose, hemicellulose, and lignin in rice straw, a governing equation was developed to describe the variation of organic acid concentration over time during a fermentation process run on mixed raw materials.
2. Accordingly, the optimal ratio of duck dung to straw in the mixture was 2.8:1 when generating acid and methane during fermentation. Through the mathematical model and research data from the fermentation experiments, the activation energy and pre-exponential factors with respect to cellulose, hemicellulose, and lignin were deduced,

and they suggested that lignin had the highest activation energy, followed by that of hemicellulose and cellulose.

3. The results indicated that the inclusion of a suitable amount of Mn^{2+} (at a concentration of $2 \text{ mol} \times L^{-1}$) was able to strengthen MnP activity, improve the ability of white-rot fungi to rupture β -O-4 bonds, and increase the amount of gas produced.
4. In response to the *de facto* production conditions for fermentation/biogas production *via* the duck dung and straw mixture, a modification pre-treatment *via* activated carbon-based solid acid (for 6 d) was conducted before rice straw fermentation. The results showed that the experimental group generated 15.8% more cumulative biogas than the control group. The gas yield reached its peak when the pre-treatment benefitted from the addition of 300 g of inoculum at a concentration of 30%.

ACKNOWLEDGMENTS

This project was supported by the Key Program of Henan Province Higher Education Institutions (19A480007), the Changzhou University Fund Project (ZMF19020301), and the Research Business Special Fund Project for Zhongyuan University of Technology (K2018YY005) of China.

REFERENCES CITED

- Ai, P., Zhang, Y. L., Sheng, K., Zhai, H., and Yan, S. P. (2010). "Pretreatment for biogas production by anaerobic fermentation of rice straw," *Transactions of the Chinese Society of Agricultural Engineering* 26(7), 266-271. DOI: 10.3969/j.issn.1002-6819.2010.07.046
- Braber, K. (1995). "Anaerobic digestion of municipal solid waste: A modern waste disposal option on the verge of breakthrough," *Biomass and Bioenergy* 9(1-5), 365-376. DOI: 10.1016/0961-9534(95)00103-4
- Buswell, A. M., and Hatfield, W. D. (1936). "Laboratory studies of sludge digestion," *Urbana: State of Illinois Department of Registration and Education* 32, 25-32.
- Chen, C. L., Yang, Y., Hu, L., and Xie, G. H. (2016). "Review on the development of crop residue management policies in provincial regions in China," *Journal of China Agricultural University* 22(11), 1-16. DOI: 10.11841/j.issn.1007-4333.2017.11.01
- Chen, H. Z., and Li, S. Z. (2002). "Lignocellulosical microorganisms and biomass total utilization," *Biotechnology Bulletin* 2002(2), 25-29, 34. DOI:10.3969/j.issn.1002-5464.2002.02.006
- Chen, L., Zhao, L. X., Dong, B. C., Wan, X. C., and Gao, X. X. (2010). "The status and trends of the development of biogas plants for crop straws in China," *Renewable Energy Resources* 28(3), 145-148. DOI:10.13941/j.cnki.21-1469/tk.2010.03.027
- Chu, J. Y., and Huang, Y., and Zhao, Q. (2017). "Analysis of the mesophilic anaerobic fermentation characteristics of pig manure mixed with the fresh sweet potato vine in different ratios," *Journal of Safety and Environment* 17(6), 2366-2370. DOI:10.13637/j.issn.1009-6094.2017.06.061

- Chu, L. L. (2011). *Conditions and Biogas Production Effect of Efficient Biogas Production Anaerobic Fermentation*, Ph.D. Dissertation, Yangling, ShanxiNorthwest A&F University.
- Dan, L., and Wei, G. (2010). "Research progress of application of biogas dry fermentation technology in livestock and poultry manure treatment," *Shandong Journal of Animal Science and Veterinary Medicine* 31(4), 67-69. DOI:10.3969/j.issn.1007-1733.2010.04.043
- Fan, P. C., Tian, J., Huang, J. M., Lei, W. Q., and Qiu, H. D. (2008). "On the determination of cellulose and lignin of peanut shells," *Journal of Chongqing University of Science and Technology* 10(5), 64-67. DOI:10.3969/j.issn.1673-1980.2008.05.020
- Feng, L., Li, R. D., and Li, Y. J. (2010). "Effects of straw pretreated with sodium hydroxide at solid state on biogasification efficiency in its anaerobic digestion," *Journal of Shenzhen University Science and Engineering* 27, 367-373.
- Gao, B., Chang, Z., Ye, X., Du, J., Xu, Y., and Zhang, J. (2010). "Effect of compost pretreatment on biogas production from rice straw," *Transactions of the Chinese Society of Agricultural Engineering* 26(5), 251-256. DOI: 10.3969/j.issn.1002-6819.2010.05.043
- Guo, P., Mochidzuki, K., Cheng, W., Zhou, M., Gao, H., Zheng, D., Wang, X., and Cui, Z. (2011). "Effects of different pretreatment strategies on corn stalk acidogenic fermentation using a microbial consortium," *Bioresource Technology* 102(16), 7526-7531. DOI: 10.1016/j.biortech.2011.04.083
- Hu, X. M., Zhang, W. D., Yin, F., Liu, S. Q., Li, J. C., Xu, R., and Chen, Y. B. (2010). "Experimental study of biogas fermentation with rice straw pretreated by microbe," *Journal of Anhui Agricultural Sciences* 38(23), 12797-12799. DOI: 10.3969/j.issn.0517-6611.2010.23.179
- Keller, F. A., Hamilton, J. E., and Nguyen, Q. (2003). "Microbial pretreatment of biomass: Potential for reducing severity of thermochemical biomass pretreatment," *Applied Biochemistry and Biotechnology* 105(1-3), 27-41. DOI: 10.1385/ABAB:105:1-3:27
- Lange, M., and Ahring, B. K. (2001). "A comprehensive study into the molecular methodology and molecular biology of methanogenic," *Archaea FEMS Microbiol Rev* 25(5), 553-571. DOI:10.1111/j.1574-6976.2001.tb00591.x
- Li, L. H., Ma, L. L., Yuan, Z. H., Liu, X. F., and Liao, C. P. (2007). "Study on anaerobic digestion of straw stalk," *Journal of Agro-Environment Science* 26(1), 335-338. DOI:10.3321/j.issn:1672-2043.2007.01.068
- Li, S. Y. (2017). "Study on biomass energy conversion technology and resources comprehensive development and utilization," *China Resources Comprehensive Utilization* 35(10), 46-47, 71. DOI:10.3969/j.issn.1008-9500.2017.10.018
- Li, Y. B., Bai, J. R., Yang, G. H., Li, Y., Guo, O. Y., and Ren, G. X. (2009). "Influence factors of biogas yields," *Journal of Northwest A&F University* 37(5), 171-177. DOI:10.13207/j.cnki.jnwafu.2009.05.003
- Liu, E., and Liu, S. (2017). "Process optimization and study of biogas fermentation with a mixture of duck manure and straw," *Renewable and Sustainable Energy Reviews* 72, 439-444. DOI: 10.1016/j.rser.2017.01.045
- Liu, E., Liu, S., Bai, B., and Chen, P. (2013). "Development of dynamic model of cornstalk bale combustion," *Transactions of the Chinese Society of Agricultural Engineering* 29, 218-226. DOI: 10.3969/j.issn.1002-6819.2013.24.029

- Liu, J. H., Wang, G. H., Zhang, B. L. (2006). "Reasonable thought on the industrialization of straw densification briquette fuel," *Transactions of the CSAE* 22(S1), 138-141.
- Liu, S., Wang, Y., Bai, B., Su, C., Yang, G., and Zhang, F. (2011). "Analysis on combustion kinetics of corn stalk briquetting densification fuel," *Transactions of the Chinese Society of Agricultural Engineering* 27(9), 287-292. DOI: 10.3969/j.issn.1002-6819.2011.09.050
- Liu, Y. (2015). *Study on Cellulose Hydrolysis Using Carbon-based Solid Acid Catalyst*, Master's Thesis, Beijing, North China Electric Power University. DOI: 10.7666/d.Y2879225
- Mao, G., Zou, H., Chen, G., Du, H., and Zuo, J. (2015). "Past, current and future of biomass energy research: A bibliometric analysis," *Renewable and Sustainable Energy Reviews* 52, 1823-1833. DOI: 10.1016/j.rser.2015.07.141
- Metha, V., Gupta, J. K., and Kaushal, S. C. (1990). "Cultivation of *Pleurotus florida* mushroom on rice straw and biogas production from the spent straw," *World Journal of Microbiology and Biotechnology* 6, 366-370. DOI: 10.1007/BF01202116
- Müller, H. W., and Trösch, W. (1996). "Screening of white rot fungi for biological pretreatment of wheat straw for biogas production," *Applied Microbiology and Biotechnology* 24, 180-185. DOI: 10.1007/BF01982565
- Song, L. (2013). "Mechanism study on improvement of enzymatic hydrolysis of corn stover by efficient lignin modification with white-rot fungus," Wuhan, Huazhong University of Science & Technology (PhD Thesis, China Doctoral Dissertations Full-text Database). DOI:10.7666/d.D409487
- Song, Z. L., Li, Y. B., Yang, G. H., Qin, J. J., Ren, G. X., and Feng, Y. Z. (2010). "Effects of total solid concentration and temperature on biogas yields of mixture of chicken manure and corn straw," *Transactions of the Chinese Society of Agricultural Engineering* 26(7), 260-265. DOI: 10.3969/j.issn.1002-6819.2010.07.045
- Song, Z. L., Guo, Y., Yang, G. H., Ren, G. X., Feng, Y. Z., and Zhou, X. W. (2012). "Factors affecting biogas yields of manure-straw mixture," *Journal of Agro-Environment Science* 31(8), 1624-1629.
- Svensson, L. M., Christensson, K., and Björnsson, L. (2005). "Biogas production from crop residues on a farm-scale level: Is it economically feasible under conditions in Sweden?," *Bioprocess and Biosystems Engineering* 28, 139-148. DOI: 10.1007/s00449-005-0016-1
- Talebna, F., Karakashev, D., and Angelidaki, I. (2010). "Production of bioethanol from wheat straw: An overview on pretreatment hydrolysis and fermentation," *Bioresource Technology* 101(13), 4744-4753. DOI: 10.1016/j.biortech.2009.11.080
- Tuomela, M., Vikman, M., Hatakka, A., and Itävaara, M. (2000). "Bio-degradation of lignin in a compost environment: A review," *Bioresource Technology* 72(2), 169-183. DOI: 10.1016/S0960-8524(99)00104-2
- Wang, C., Li, D., Wang, F., and Yang, Q. (2006). "Experimental study on the combustion characteristics of biomass pellets," *Transactions of the Chinese Society of Agricultural Engineering* 22, 182-185.
- Wang, C. Y. (2015). "Research on heterogeneous hydrolysis of cellulose with carbon-based solid acid prepared from cellulose and lignin," Taiyuan, Taiyuan University of Technology (Master Thesis).

- Wang, F., Cai, Y. Q., and Qiu, H. G. (2012). "Current status, incentives and constraints for future development of biogas industry in China," *Transactions of the CSAE* 28(1), 184-189. DOI:10.3969/j.issn.1002-6819.2012.01.033
- Yan, Z. Y., Yuan, Y. X., Liu, X. F., Liao, Y. Z., and He, R. N. (2009). "A study on biogas fermentation with straws," *Journal of Sichuan Agricultural University* 27(2), 176-179. DOI:10.3969/j.issn.1000-2650.2009.02.010
- Yang, L., Zhang, T., Wang, Y. Z., and Wang, J. H. (2008). "Comparison on the biogas production effect of different stalks anaerobic fermentation," *Renewable Energy Resources* 26(5), 46-52. DOI:10.13941/j.cnki.21-1469/tk.2008.05.017
- Yang, T. X. (2014). "Conversional mechanism and microbial regularity analysis of corn stover during dry anaerobic digestion," Wuhan University, Wuhan, China (PhD Thesis, China Doctoral Dissertations Full-text Database).
- Yu, F. B., Luo, X. P., Guan, L. B., Zhang, M. X., and Shan, S. D. (2008). "Research advance in biogas fermentation microorganism," *Journal of Anhui Agricultural Sciences* 36(35), 15658-15660. DOI: 10.3969/j.issn.0517-6611.2008.35.152
- Zhang, C. L., Yang, G. H., Bu, D. S., Chu, L. L., Ben, G. X., and Feng, Y. Z. (2008). "Effect of temperature on biogas production and fermentation period length from the anaerobic digestion of crop residue," *Journal of Agro-Environment Science* 27(5), 2069-2074. DOI:10.3321/j.issn:1672-2043.2008.05.069
- Zhang, X. L., Yue, L., Chai, Q. M., Zhang, C. L. (2006). "Foreign policies for exploitation and utilization of biomass energy," *Transactions of the Chinese Society of Agricultural Engineering* 22(S1), 4-7.
- Zhou, W., Dong, B. C., and Qi, Y. (2010). "The gas productions of different treated straw under mesophilic and anaerobic condition," *China Biogas* 28(3), 22-24. DOI: 10.3969/j.issn.1000-1166.2010.03.006

Article submitted: April 18, 2020; Peer review completed: September 27, 2020; Revised version received: October 5, 2020; Accepted: October 6, 2020; Published: October 9, 2020.

DOI: 10.15376/biores.15.4.8862-8882