A biologically inspired variable-pH strategy for enhancing short-chain fatty acids (SCFAs) accumulation in maize straw fermentation

A biologically inspired variable-pH strategy for enhancing short-chain fatty acids (SCFAs) accumulation in maize straw fermentation

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Abstract

This study investigates the feasibility of varying the pH to enhance the accumulation of short-chain fatty acids (SCFAs) in the in vitro fermentation of maize straw. The corresponding hydrolysis rate and the net SCFA yield increased as inoculum ratio ($VS_{inoculum}/VS_{substrate}$) increased from 0.09 to 0.79. The pH were maintained at 5.3, 5.8, 6.3, 6.8, 7.3, and 7.8, respectively. A neutral pH of approximately 6.8 was optimal for hydrolysis. The net SCFA yield decreased by 34.9% for a pH of less than 5.8, but remained constant at approximately 721±5 mg/g_{vs} for a pH between 5.8 and 7.8. In addition, results were obtained for variable and constant pH levels at initial substrate concentrations of 10, 30 and 50 g/L. A variable pH increased the net SCFA yield by 23.6%, 29.0%, and 36.6% for concentrations of 10, 30 and 50 g/L. Therefore, a variable pH enhanced SCFA accumulation in maize straw fermentation.

Keywords: SCFAs production; Maize straw; pH; Anaerobic digestion; Rumen.

1 Introduction

Short-chain fatty acids (SCFAs) can be used as an organic carbon source for nitrogen and phosphorus removal in wastewater treatment plants or to produce biogas, hydrogen, electricity, biodiesel, and bioplastic polyhydroxyalkanoates (PHAs) (Lee et al., 2014). Most solid wastes e. g. sludge (Ji et al., 2010), food waste (Kim et al., 2006), and municipal solid waste (Bolzonella et al., 2005) can be used as source materials for SCFAs production. Hence, the production of SCFAs from solid wastes has recently

attracted increasing attention from the research community. Because of the high efficiency of rumen in which the organic loading rate (OLR) can be greater than 100 $g_{vs}/(L \cdot d)$ (Meng et al., 2013), a lot of studies have been conducted on producing SCFAs with the mechanisms of ruminant digestive systems. These studies took three approaches. One approach was to examine the fermentation process at the molecular level to understand the high degradation efficiency of rumen microorganisms processing lignocellulosic wastes (Hu et al., 2008). However, rumen microorganisms are difficult to obtain and do not remain active in vitro for extended periods of time (Chapleur et al., 2014); thus, this approach is not practical. The second approach was to represent the alimentary canals of various species of animals as sets of processes, such as various types of reactors (Godon et al., 2010). The third approach was to construct an artificial rumen (RUSITEC) (Czerkawski and Breckenridge, 1977), but this method has mainly been used to study ruminant digestion. Several modified RUSITEC systems have been used to study the decomposition of lignocellulosic waste materials (Gijzen et al., 1986), paper mill sludge (Gijzen et al., 1988), and cereal residues (Kivaisi et al., 1992). However, few studies have investigated the process in depth or on a large scale. Each study focused on one aspect, and there was not a sufficiently comprehensive analysis of the mechanisms of ruminant digestive systems that would explain their high efficiency.

According to the authors' analysis, the mechanisms of high efficiency of ruminant digestive systems can be summarized as three aspects. Firstly, the high efficiency of rumens can be attributed to the special microbial communities that they contain, which include bacteria, fungi, archaea, and protozoa (Liu, 1991). Future research should focus on maintaining an in vitro environment similar to that in a rumen to support the activity of rumen microorganisms but not inoculate them directly. Secondly, the processes and conditions particular to rumens, such as immediate product removal, precise salivation, rumination, rumen peristalsis, a constant temperature, and the special pH condition, are all possible mechanisms that can enhance fermentation. Thirdly, the well-organized interactions of the four chambers in the stomachs of ruminants (the rumen, reticulum, omasum, and abomasum) contribute to the fermentation process.

An example of the mechanisms is the special pH condition. The difference between natural rumen and artificial systems is that SCFA production and salivation cause the pH in the rumen to vary between approximately 5.5 and 7.0 (Feng, 2004), unlike in artificial fermentation digesters, in which the pH remains relatively constant. Fermentation can be significantly influenced by pH (Wu et al., 2009). A neutral pH is optimal for most microorganisms, increasing product consumption (Elango et al., 2007). Product consumption can be reduced by lowering the pH, but hydrolysis and acidogenesis may also be inhibited because the growth or activity of the ruminal bacteria would be reduced (Russell and Rychlik, 2001; Sari et al., 2015). The activities

of some key enzymes for SCFA forming at higher pH were higher than those at neutral or acidic pH (Zhao et al., 2015), however, it needs alkali addition. Therefore, fermentation with a variable pH, such as what occurs in a rumen, could potentially enhance SCFA accumulation. Until now, few studies on the effect of a variable pH condition on fermentation were reported.

This research investigates the effects of a variable pH level on the in vitro fermentation of maize straw to inform further research in which the process will be sustained. Additionally, the potential of SCFA production from maize straw and the effects of the inoculum ratio and pH on maize straw fermentation are investigated.

2 Materials and Methods

2.1 Substrates and inoculum properties

Maize straw, a kind of source material of fodder for ruminants, was used as substrate in this study. It was obtained from the China Agricultural University, Shangzhuang experimental farm in Beijing, China. Following harvesting, the straw was chopped with a chaff cutter (Taifeng, Qufu, China) and then milled in a straw pulverizer (Yijian, Jinan, China) to the fineness of a #50 mesh. The pulverized straw was stored in a sealed bottle at room temperature. Prior to use, the pulverized straw was air-dried until the moisture content was 0% at 105°C .

Rumen fluid, which contains few methanogens and is considered suitable for SCFA production, was used as the inoculum in this study. Three samples of the fluid were

obtained from each of three milk cows at the China Agricultural University, Beijing, China. The fluid samples were filtered through four layers of gauze and then stored in a thermos bottle. The fluid samples were used in the experiments within 3 h of being drawn from the donor animals. The properties of the substrate and inoculums are provided in Table 1.

2.2 Experimental setup and operation

Three experiments were conducted in this study. In experiment A, samples were prepared in which 3.75 g of pulverized maize straw was inoculated with 25, 75, 125, 175, and 225 mL of rumen fluid. In addition, 150 mL of artificial saliva and deionized water were added to achieve a total working volume of 375 mL. The pH was maintained at 6.8. In experiment B, the pH was maintained at values 5.3, 5.8, 6.3, 6.8, 7.3, and 7.8. Each sample consisted of 3.75 g of pulverized straw, 200 mL of rumen fluid, and 175 mL of artificial saliva. In experiment C, the pH was allowed to vary, i.e., decrease naturally, in certain samples (V-10, V-30, and V-50), and the pH in the remaining samples (C-10, C-30, and C-50) was held constant at 6.8. The amounts of pulverized maize straw used in samples V-10, V-30, and V-50 were 3.75, 11.25, and 18.75 g, respectively. Similarly, 3.75, 11.25, and 18.75 g of pulverized maize straw were used in the constant-pH samples C-10, C-30, and C-50, respectively. To each sample, 125 mL of rumen fluid and 250 mL of artificial saliva were added. Replicate samples were prepared in all three of the experiments.

All of the samples were prepared in 500 mL serum bottles. The working volume was 375 mL. All of the bottles were placed in an incubator at a temperature of 39.0±0.5°C and stirred at a rate of 100 rpm. The pH was controlled by a system that automatically meted a sodium hydroxide solution (1 mol/L). The pH control system included a pH sensor (Mettler-Toledo, Switzerland), a pH controller (ARK 82, China), and a peristaltic pump (Model BQ50-1J, Baoding Longer Precision Pump Co., Ltd., China). The pH data were recorded automatically by an electronic recorder (Weimingshouwang SY2000C, China). All tests were conducted for 72 h, which was sufficiently long to complete the acidification process (Paul et al., 2011). The samples were analyzed at the end of the 72 h period. Prior to the start of the experiments, the seal on each bottle was tested for gas and liquid leakage. The artificial saliva was prepared according to the procedure of Menke et al (1988).

2.3 Analytical methods

The gas yield was measured using water volume replacement methods. The total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD) were determined using APHA standard methods (APHA, 1998). The SCFAs were measured with a gas-phase chromatograph (Model 7890A, Agilent, USA) equipped with a $30\mathrm{m}\times\Phi 0.53\mathrm{mm}\times 1.0\mu\mathrm{m}$ capillary column (Model DB-FFAP, Agilent, USA) and flame ionization detectors; the operating temperature was 230°C.

The operating temperature of the oven was held at 70°C for 3 min, increased to 180°C at a rate of 20°C/min, and held at 180°C for 5 min. Nitrogen was used as the carrier gas. The fractions of C, H, and N were analyzed with an elemental analyzer (Model CE-440, EAI, USA). The gas composition was measured with the gas-phase chromatograph fitted with a thermal conductivity detector and a 4.5 m × 2 mm #60/80-mesh capillary column (Carboxen 1000, Agilent, USA). Argon was used as the carrier gas at a flow rate of 30 mL/min. The temperatures of the injector, detector, and column were 150, 250 and 150°C, respectively.

2.4 Calculations

The hydrolysis rate was determined from the VSS conversion rate, as shown in Eq. (1).

$$\eta_H = \left(1 - \frac{m_t}{m_0}\right) \cdot 100\% = \left(1 - \frac{V_t \cdot c_{VSS} - V'_t \cdot c'_{VSS}}{m_{\text{substrates}} \cdot VS}\right) \cdot 100\% \quad (1)$$

In Eq. (1), η_H is the hydrolysis rate, which is equal to the VSS conversion rate, m_t is the mass of the VS in the substrate remaining in the system after fermentation, m_0 is the mass of the VS in the substrate added at the beginning of the fermentation test (because the sodium hydroxide solution was added to control the pH and evaporation, the working volume changed during the experiments), V_t and c_{VSS} are the final working volume and final VSS concentration of the fermentation system, respectively, V'_t and c'_{VSS} are the final working volume and final VSS concentration of the fermentation system of the blank group, respectively, $m_{\text{substrates}}$ is the mass of the straw, and VS is the percentage of VS in the straw.

Acidogenesis occurs following hydrolysis; thus, the reactants for acidogenesis are the products of hydrolysis. To assess the acidogenesis process, the net SCFA yield can be expressed as in Eq. (2).

$$r_{SCFAs} = \frac{m_{SCFAs}}{m_0} \cdot \eta_H = \frac{V_t \cdot c_{\mathrm{SCFAs,t}} - V_0 \cdot c_{\mathrm{SCFAs,0}}}{m_{\mathrm{substrates}} \cdot VS} \cdot \eta_H \quad (2)$$

where r_{SCFAs} is the net SCFA yield, m_{SCFAs} is the mass of SCFAs produced, m_0 is the mass of the VS in the substrate added at the beginning of the fermentation test, η_H is the hydrolysis rate, V_{t} and V_0 are the final and initial working volumes of the system, respectively, $c_{\mathrm{SCFAs,t}}$ and $c_{\mathrm{SCFAs,0}}$ are the final and initial total SCFA concentrations of the system, respectively, $m_{\mathrm{substrates}}$ is the mass of the pulverized straw, and VS is the percentage of VS in the straw.

It is difficult to make the exact detail reactions which occur during fermentation clear. In order to calculate the mass balance, the fermentation was simplified on the assumption that only cellulose and hemi-cellulose are hydrolyzed. Although the cellulose and hemi-cellulose content of the substrates are known, the ratio of pentose and hexose in hemi-cellulose was not clear. However, the result must be between the values assuming that all the hydrolyzed products are pentose or hexose, respectively. Therefore, the average value on the assumption that either pentose or hexose were the sole hydrolyzed products was used for mass balance calculation. The hydrolysis reactions are shown in Eq. (3) and (4).

$$(C_5H_8O_4)_n + \mathrm{n}H_2\mathrm{O} \longrightarrow \mathrm{n}C_5H_{10}O_5 \quad (3)$$

$$(C_6H_{10}O_5)_n + \mathrm{n}H_2\mathrm{O} \longrightarrow \mathrm{n}C_6H_{12}O_6 \quad (4)$$

The acidogenesis reactions of pentose or hexose are shown in Eq. (5) – (12).

$$C_5H_{10}O_5 + H_2\mathrm{O} \longrightarrow 2CH_3COOH + CO_2 + 2H_2 \quad (5)$$

$$C_5H_{10}O_5 + H_2\mathrm{O} \longrightarrow CH_3CH_2COOH + 2CO_2 + 3H_2 \quad (6)$$

$$C_5H_{10}O_5 \longrightarrow CH_3CH_2CH_2COOH + CO_2 + H_2O \quad (7)$$

$$C_5H_{10}O_5 + 3H_2 \longrightarrow CH_3CH_2CH_2CH_2COOH + 3H_2O \quad (8)$$

$$C_6H_{12}O_6 + 2H_2\mathrm{O} \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (9)$$

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_2COOH + 2H_2O \quad (10)$$

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (11)$$

$$C_6H_{12}O_6 + H_2 \longrightarrow CH_3CH_2CH_2CH_2COOH + CO_2 + 2H_2O \quad (12)$$

The methanogenesis was ignored because little methane was detected. The H_2O for hydrolysis reaction and solid residues were calculated according to the hydrolyzed TS. The H_2O for acidogenesis reaction, SCFA, CO_2, and H_2 were calculated according to produced SCFA.

3 Results and Discussion

3.1 Effect of the inoculum ratio

To account for differences in the inoculum ratio in experiment C, the effect of the inoculum ratio on fermentation was investigated in experiment A. The inoculum ratios in experiment A were 0.09, 0.26 0.44, 0.62 and 0.79 for rumen fluid volumes of 25, 75, 125, 175, and 225 mL, respectively. As shown in Fig. 1, the hydrolysis rate increased from 48±4% to 84±2% as the inoculum ratio increased from 0.09 to 0.79. Zhou et al. (2011) reported that the reduction in VS in fresh okara (soybean curd refuse or residue) increased from 24.3% to 52.5% as the inoculum ratio increased from 0.33 to 1.00. Gunaseelan (1995) reported that the reduction in VS in parthenium increased from 23.3% to 40.9% as the inoculum ratio increased from 40.4 to 202.0 $mL_{inoculum}/g_{substrate}$. In this study, the net SCFA yield increased from 248±29 to 710±34 mg/g_{vs} and the gas yield increased from 26 to 153 mL as the inoculum ratio increased (Fig. 1). The ratio of microorganisms to the VS in the substrate and the enzyme concentration increased as the inoculum ratio increased, which increased the reaction rate, and thus, the higher inoculum ratio enhanced hydrolysis and acidogenesis. The fermentation potential of this amount of substrate could be explored by increasing the inoculum ratio.

3.2 Effect of different constant pH

To compare the results of the constant-pH and variable-pH samples in experiment C, the effect of pH on fermentation was investigated in experiment B. The hydrolysis rate,

net SCFA yield, and gas yield are shown in Fig. 2. The highest hydrolysis rate was 69±7% at pH 6.8. When the pH was maintained at an alkaline pH of 7.8, the hydrolysis rate decreased to 45±6%. When the pH was maintained at an acidic pH of 5.3, the hydrolysis rate decreased to 38±7%. Therefore, a neutral pH of approximately 6.8 is the optimal pH for maize straw hydrolysis. Hu et al. (2004) found that cellulose inoculated with rumen cultures degraded fastest when the pH was 6.8. In research by Hu and Yu (2006) on anaerobic digestion of cattails with rumen cultures, the highest VS conversion efficiency, 66%, was achieved at pH 6.7, which is similar to value obtained in this study. Lignocellulosic materials, such as maize straw, consist of cellulose and hemicellulose, which are cross-linked and strongly bound to lignin (Gu et al., 2015). This complex structure severely restricts enzymatic and microbial accessibility, and thus, the conversion rate and the reaction speed of fermentation are limited (Pu et al., 2013). Fiber digestion is a pH-sensitive process (Sari et al., 2015), and the reduction in fiber digestion at lower pH levels is likely the result of a reduction in the growth or activity of the ruminal cellulolytic bacteria (Russell and Rychlik, 2001).

In contrast, the net SCFA yield remained nearly constant at approximately 721±5 mg/g_{vs} for a pH between 5.8 and 7.8. The net SCFA yield was only 470±25 mg/g_{vs} at a pH of 5.3, which was 34.9% lower than that of the other samples (pH = 5.8-7.8; Fig. 2). These results indicate that pH levels from 5.8 to 7.8 have only a slight influence on

acidogenesis, and pH levels below 5.8 inhibit acidogenesis. The acidogenesis bacteria tolerate a wider range of pH than the hydrolysis bacteria (Ren and Wang, 2004). However, certain types of ruminal bacteria are sensitive to pH, and their activity can be inhibited if the pH is lower than 6.0 (Russell and Rychlik, 2001). A pH of less than 6.0 promotes lactic acid production (Zhao et al., 2015). The main products of digestion by rumen microorganisms are acetic, propionic, and butyric acids (Liu, 1991). The SCFA composition was not significantly influenced by the pH level when the pH was held constant. Acetic acid, propionic acid, and n-butyric acid comprised approximately 50%, 20%, and 20% of the total SCFAs. At pH 5.3, the activity of the rumen microorganisms responsible for acidogenesis, which mainly produces acetic, propionic, and n-butyric acid, was inhibited.

The gas yields were 63, 143, 135, 125, 106, and 71 mL as the pH increased from 5.3 to 7.8. The gas yield at pH 5.8 was far higher than that at pH 5.3. The gas yield decreased monotonically as the pH increased from 5.8 to 7.8. The gas volume detected was not the actual volume of gas produced because of the higher solubility of CO_2 in higher-pH solutions; i.e., more CO_2 was dissolved in and less was released from the samples with higher pH levels.

3.3 Effect of variable pH

The pH levels of the constant-pH samples (C-10, C-30, and C-50) were held constant at 6.8 during the 72 h experiments. The time histories of pH in the variable-pH samples

(V-10, V-30, and V-50) are shown in Fig. 3. The pH decreased as fermentation progressed. The final values of pH for the V-10, V-30, and V-50 samples were 6.54±0.12, 5.84±0.08, and 5.31±0.18, respectively, and the corresponding final values of the SCFAs concentration were 8,402±49, 13,605±244, and 17,556±50 mg/L, respectively. In the samples with higher initial substrate concentrations, more substrate was fermented, more H^+ was produced, the final values of pH were lower, and the final values of SCFAs concentration were higher.

The pH time histories of the variable-pH samples were linear during the first several hours. The value of the correlation coefficient r^2 for pH and time was greater than 0.9900 for all of the samples, as shown in Table 2. The slopes of the pH curves during this period for the V-10, V-30, and V-50 samples were -0.0420, -0.0633, and -0.1063, respectively. The absolute values of the slope were higher in the samples with higher initial substrate concentrations. This result indicates that the pH decreased faster in the samples with higher initial substrate concentrations. Similar results were observed in a previous study (Meng et al., 2013). The inhibition of fermentation products was not as significant during the first several hours. The reaction speed in the samples with higher initial substrate concentrations was higher because of the greater amounts of substrate present. The fermentation potential of the amount of inoculum used in experiment C could be explored by increasing the substrate concentration (10-50 g/L, inoculum ratio: 0.32-0.06). There were two main differences between the constant-pH and variable-pH

samples. One difference was that the average pH levels in the variable-pH samples were lower than that in the constant-pH samples (6.8). The other difference was that the pH was not constant in the variable-pH samples, as is the case in an actual rumen.

The hydrolysis rates for the constant-pH and variable-pH samples are shown in Fig. 4. The hydrolysis rates of the constant-pH samples (55±2%, 51±5%, and 37±3%) were slightly higher than those of the variable-pH samples (49±7%, 38±1%, and 27±1%). As noted previously, a neutral pH of approximately 6.8 was the optimal value for maize straw hydrolysis. The average pH levels of the variable-pH samples were lower than those of the constant-pH samples. However, there was little indication that activity was inhibited, especially in the samples with lower initial substrate concentrations.

The hydrolysis rates decreased as initial substrate concentration increased in both the constant-pH and variable-pH samples. The same amounts of inoculum were used, and the inoculum ratio decreased from 0.32 to 0.06 as the initial substrate concentration was increased from 10 to 50 g/L. As stated previously, higher inoculum ratios resulted in higher hydrolysis rates. The hydrolysis rate decreased as the initial substrate concentration increased in this experiment.

As shown in Fig. 5, the net SCFA yields of the variable-pH samples (983±13, 894±23, and 1104±5 mg/g_{vs}) were 23.6%, 29.0%, and 36.6% higher than those of the constant-pH samples (795±5, 693±10, and 808±31 mg/g_{vs}) for initial substrate concentrations of 10, 30, and 50 g/L, respectively. The average of the net SCFA yield for an inoculum

ratio of 0.79 in experiment A (where the pH was held constant at 6.8) and samples with pH levels from 5.8 to 7.8 in experiment B (where the pH was held constant at values of 5.8 to 7.8) were 710±34 and 721±5 mg/g_{vs}, which were also lower than those of the variable-pH samples in experiment C. Thus, the net SCFA yield for a constant pH reached a maximum value of approximately 800 mg/g_{vs}. However, the net yield for a variable pH increased to approximately 1,100 mg/g_{vs}. As previously stated, there were two differences between the constant-pH and variable-pH samples. It was demonstrated that a constant pH greater than 5.8 does not influence the net SCFA yield and acidogenesis. Therefore, the variation in pH was the reason that the net SCFA yield in the variable-pH samples was higher.

The net SCFA yields increased similarly with the initial substrate concentration in both the constant-pH and variable-pH samples. The initial substrate concentration did not significantly influence the net SCFA yield. The fermentation potential of the amount of inoculum used in experiment C could be explored by increasing the substrate concentration, as previously noted. The amount of inoculum used in each sample in experiment C was the same (125 mL of rumen fluid), and the net SCFA yield was calculated based on the hydrolyzed substrate. Furthermore, acidogenesis occurs more readily than hydrolysis (Lee et al., 2014). A fermentation time of 72 h was sufficiently long for acidogenesis to occur (Paul et al., 2011), and increasing the initial substrate concentration did not affect acidogenesis more than hydrolysis. Hydrolysis limits the

degradation rate of straw (Hu et al., 2007). It is possible that the inoculum used in experiment C contained more acidogenesis microorganisms than that used in experiment A. The net SCFA yield and acidogenesis chemical thermodynamics were similar for the various initial substrate concentrations.

Many aspects of microbial metabolism are greatly influenced by pH variations over the range within which the population of microorganisms can grow. These aspects include the utilization of carbon and energy sources, the efficiency of substrate degradation, the synthesis of proteins and various types of storage material, and the release of metabolic products from cells (Baily and Ollis, 1986). Microorganisms and the enzymes they produce typically have higher activity at a neutral pH. Moreover, pH variations can affect cell morphology and structure and therefore flocculation and adhesion (Gottschalk, 1986). The rates of hydrolysis and acidogenesis, the growth rate of the microbial population, the enzyme activity, and the product consumption rate are also higher at a neutral pH. Although acidogenesis was not inhibited at a constant pH of 6.8, the cumulative net SCFA yield was lower because of the higher product consumption rate, i.e., methanogenesis. A variable pH can provide a range in which hydrolysis would not be substantially inhibited and the product consumption rate is lower. A similar effect occurs in fruits, where a high diurnal temperature difference promotes sugar accumulation. Therefore, the pH should vary between neutral (6.8, which is optimal for

hydrolysis) and acidic (5.3 or lower, which inhibits product consumption but is not so low that microorganisms are destroyed).

The gas yields for the constant-pH and variable-pH samples are shown in Fig. 6. Only a small amount of methane was detected in the gas products, so methanogenesis can be ignored. Hu and Yu (2006) did not detect methane in the gas products in the fermentation of cattails with rumen microorganisms in the first 72 h. The gas yield curves are approximately linear for the first 12 h. The slopes of the gas yield curves during this period are similar for the constant-pH and variable-pH samples (see Fig. 6 and Table 2). Therefore, the gas production rates of both the constant-pH and variable-pH samples were similar for the first 12 h. This result indicates that the fermentation process was not significantly influenced by pH in the first 12 h. The final gas yields of the variable-pH samples were higher than those of the constant-pH samples. This result is consistent with those of VFA production shown in Fig. 5. The reason for this consistency is that most of the gas is produced from acidogenesis (Ren and Wang, 2004), so the gas yield and SCFA production are correlated. The gas production rates and final gas yields of the samples with higher initial substrate concentrations were higher because more of the substrate was present.

3.4 Mass balance

Although the final calculated mass balance (Table 3) were the average values on the assumption that either pentose or hexose were the sole hydrolyzed products, the

confidence intervals were acceptable. The trend of solid residues, SCFA, and CO_2 and H_2 mass balance was similar to the trend of hydrolysis rates, SCFA yields, and gas yields, respectively. The acidogenesis process could be reflected by the ratio of other products. The ratio of other products decreased as inoculum ratio increased in experiment A. This is because inoculum was scarce in low inoculum ratio treatments so that fermentation could not be completed in three days' time. When pH was controlled at 5.3 in experiment B, the ratio of other products was 22.9±4.2 which was obviously higher than other treatments. This is because acidogenesis process was inhibited as talked in 3.2. The ratio of other products of V-10, V-30, and V-50 (variable pH condition) were nearly 0, while other products of C-10, C-30, and C-50 (constant pH condition) were higher. This indicates that a variable pH condition indeed promotes acidogenesis process compared with constant pH condition.

Conclusions

Hydrolysis rate and net SCFA yield can be improved by higher inoculum ratio. A neutral pH of approximately 6.8 is the optimal pH for hydrolysis. pH below 5.8 inhibits acidogenesis and pH 5.8 – 7.8 does not influence acidogenesis significantly. A variable pH between neutral (6.8) and acid (5.3, or lower) pH promotes SCFA accumulation at the same time does not inhibit hydrolysis and acidogenesis process significantly. This biologically inspired variable pH strategy can be applied in other organic solid wastes fermentation under continuous conditions. This strategy provided

a new approach to improve SCFAs production in future biochemical engineering application.

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Figure Captions

Fig. 1 – Hydrolysis rate, net SCFA yield, and gas yield for various inoculum ratios.

Fig. 2 – Hydrolysis rate, net SCFA yield, and gas yield at various constant pH values.

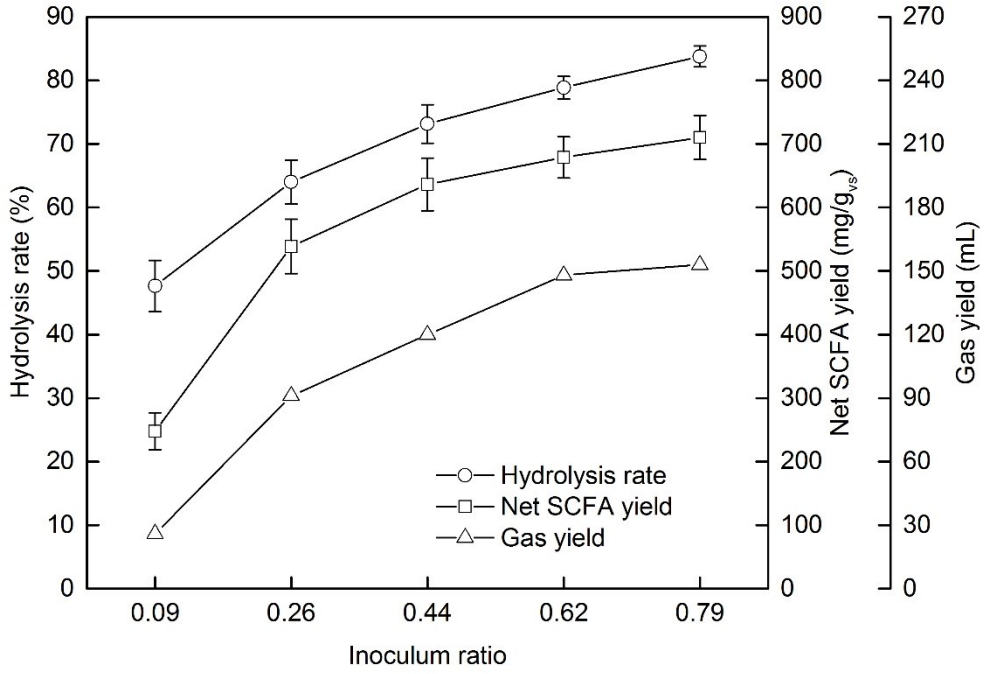
Fig. 3 – Course of pH for variable-pH samples in experiments C.

Fig. 4 – Hydrolysis rates of constant-pH and variable-pH samples in experiment C.

Fig. 5 – Net SCFA yield for the constant-pH and variable-pH samples in experiment C.

Fig. 6 – Gas yields of constant-pH and variable-pH samples in experiment C.

1 **Figures**



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3 Fig. 1 – Hydrolysis rate, net SCFA yield, and gas yield for various inoculum ratios.

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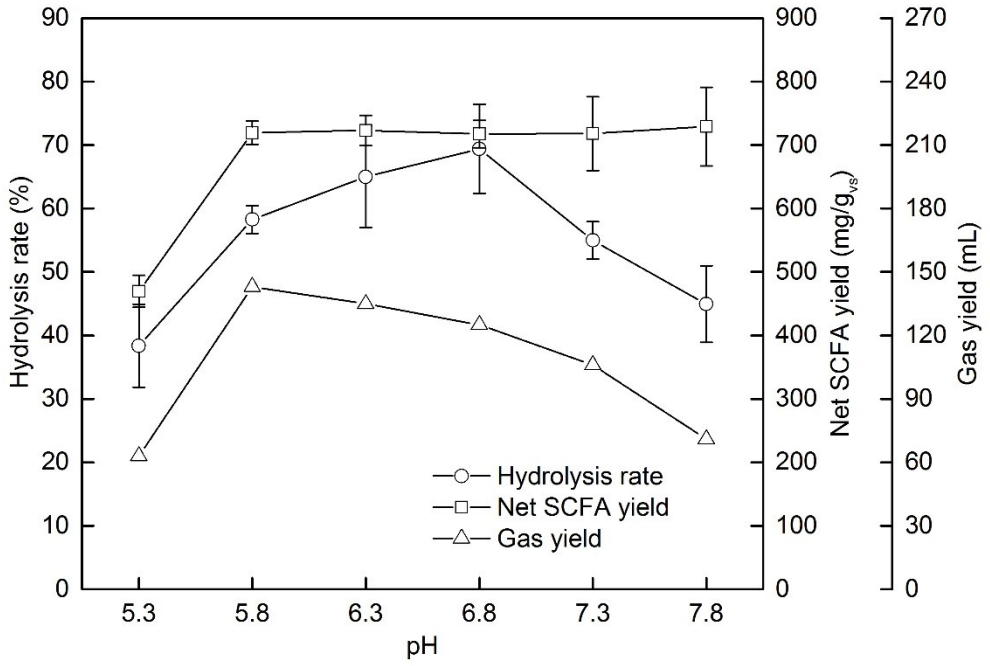

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2 Fig. 2 – Hydrolysis rate, net SCFA yield, and gas yield at various constant pH values.

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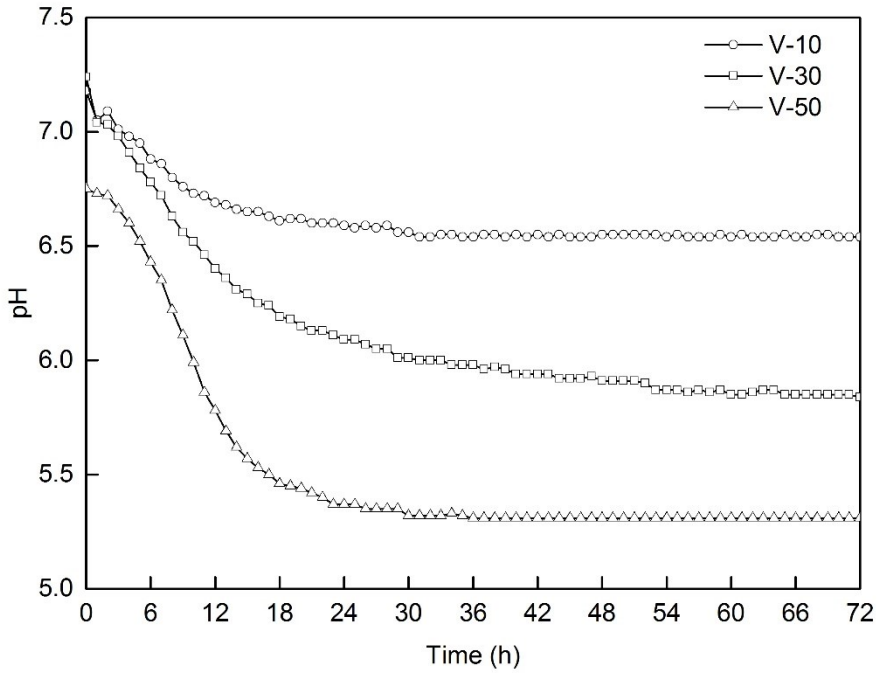

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2 Fig. 3 – Course of pH for variable-pH samples in experiments C.

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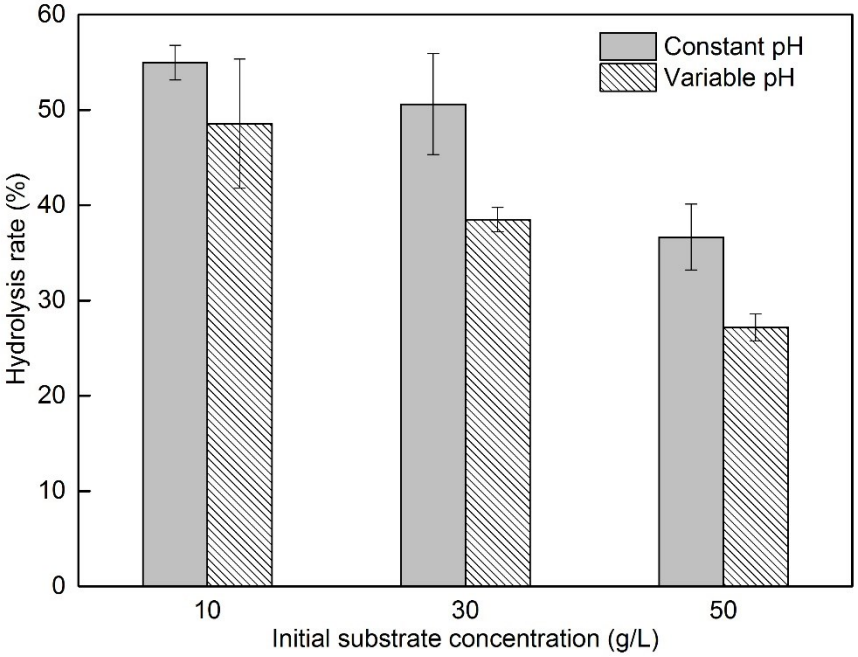

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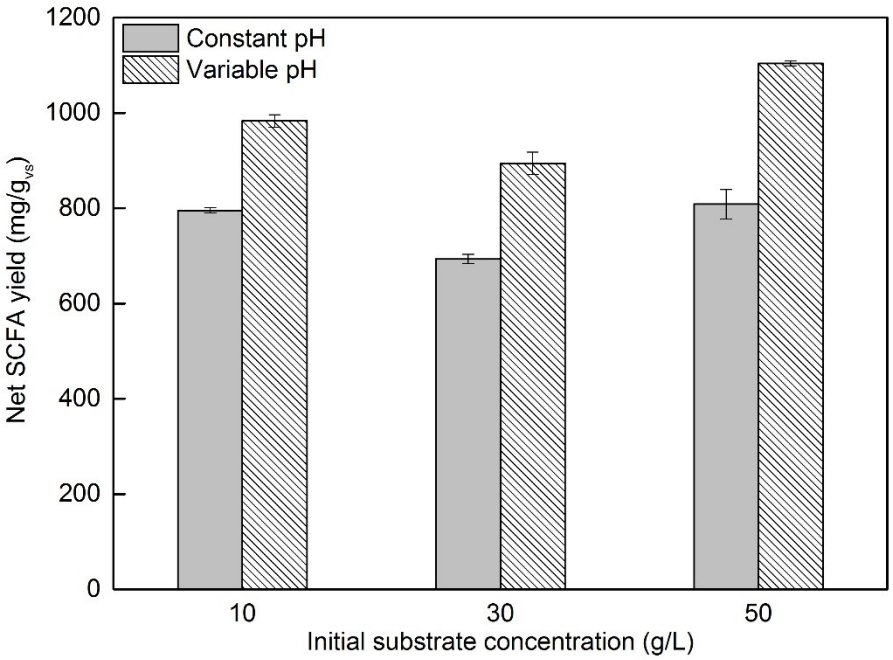
2 Fig. 4 – Hydrolysis rates of constant-pH and variable-pH samples in experiment C.

3



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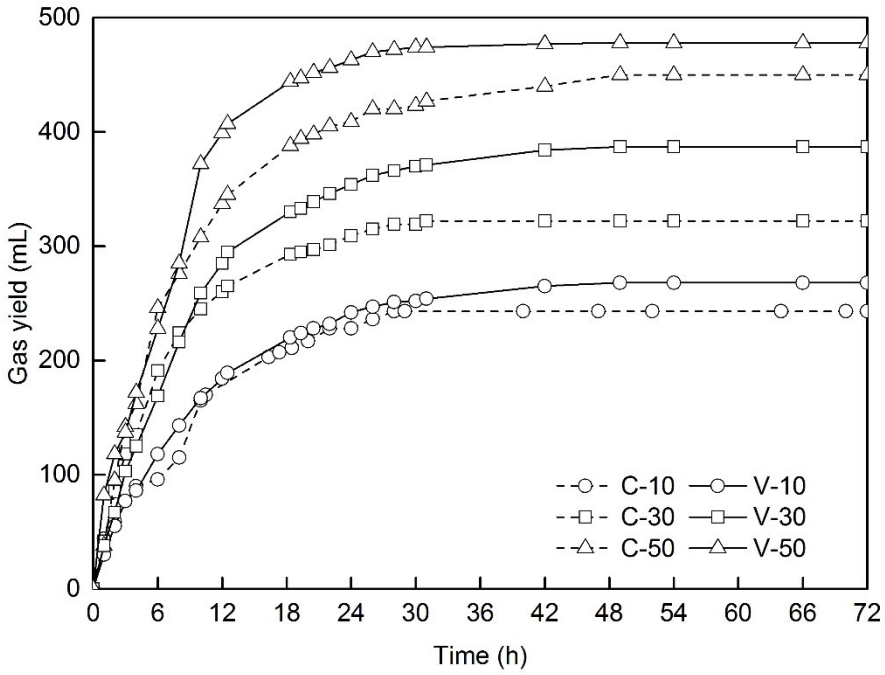
2 Fig. 5 – Net SCFA yield for the constant-pH and variable-pH samples in experiment

3 C.

4



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2 Fig. 6 – Gas yields of constant-pH and variable-pH samples in experiment C.

3

Table Captions

Table 1 – Properties of the substrate and inoculums used in the experiments.

Table 2 – pH and gas yield for constant-pH and variable-pH samples (the pH time histories are shown in Fig. 3, and the gas yield time histories are shown in Fig. 6).

Table 3 – Mass balance of all the treatments of the three experiments.

Tables

Table 1 – Properties of the substrate and inoculums used in the experiments.

Material	Component	Units	Experiment A	Experiment B	Experiment C
Straw	VS	%TS	91.73±0.34		
	C	%TS	42.42±0.14		
	H	%TS	2.16±0.04		
	N	%TS	0.59±0.09		
	Cellulose	%TS	33.25±1.97		
	Hemi-cellulose	%TS	28.47±0.65		
	Lignin	%TS	9.20±1.85		
Inoculum	TSS	g/L	14.10±1.87	9.59±0.36	11.35±0.74
	VSS	g/L	12.05±1.73	7.87±0.31	8.83±0.36
	Acetic acid	mg/L	5646±44	3621±53	5259±21
	Propionic acid	mg/L	3687±47	1406±22	2563±9
	n-Butyric acid	mg/L	3170±53	1572±2	2826±1
	Total SCFAs	mg/L	13226±157	7109±108	11292±32
	TCOD	mg/L	N.D.	23918±436	29232±631
	SCOD	mg/L	N.D.	10379±141	15869±110

Note: VS (volatile solids), TSS (total suspended solids), VSS (volatile suspended solids), Total SCFAS (total volatile fatty acids), TCOD (total chemical oxygen demand), SCOD (soluble chemical oxygen demand), N.D. (not determined).

1 Table 2 – pH and gas yield for constant-pH and variable-pH samples (the pH time

2 histories are shown in Fig. 3, and the gas yield time histories are shown in Fig. 6).

	pH			Gas yield					
Sample No.	V-10	V-30	V-50	C-10	C-30	C-50	V-10	V-30	V-50
Slope	-0.0420	-0.0633	-0.1063	21.06	27.63	35.59	21.90	26.43	32.72
r^2	0.9905	0.9952	0.9947	0.9227	0.9711	0.9717	0.9691	0.9902	0.9796

3

1 Table 3 – Mass balance of all the treatments of the three experiments.

Experiment No.	Treatments	Reactants			Products				
		Substrates	H_2O for hydrolysis reaction	H_2O for acidogenesis reaction	Solid residues	SCFAs	CO_2	H_2	Other products
A	0.09	100	5.9±1.1	1.4±0.1	45.7±3.1	9.7±0.6	5.6±0.3	0.4±0.0	46.0±3.4
	0.26	100	7.6±1.4	2.4±0.0	33.4±3.6	26.2±0.8	15.1±0.1	0.9±0.0	34.4±3.9
	0.44	100	8.5±1.6	2.3±0.2	26.2±0.8	38.2±0.9	21.9±0.5	1.2±0.0	23.4±1.9
	0.62	100	8.8±1.6	2.0±0.6	24.2±0.6	43.9±1.0	25.0±1.2	1.3±0.1	16.4±2.3
	0.79	100	8.9±1.7	1.9±0.9	23.4±0.3	48.8±0.5	27.7±1.9	1.4±0.2	9.5±2.6
B	5.3	100	5.1±0.9	1.4±1.1	52.2±3.2	14.5±0.8	16.0±2.3	0.9±0.2	22.9±4.2
	5.8	100	7.2±1.3	3.1±0.7	36.5±0.7	34.4±0.9	27.4±1.5	1.6±0.1	10.4±2.3
	6.3	100	7.6±1.4	3.4±0.7	33.0±3.3	38.6±1.4	29.7±1.6	1.7±0.1	8.0±4.2
	6.8	100	7.9±1.5	3.7±0.6	30.8±2.5	40.9±1.2	31.1±1.3	1.8±0.1	7.1±3.4
	7.3	100	6.0±1.1	3.3±0.7	45.3±1.5	32.4±2.7	26.1±1.4	1.5±0.1	4.0±3.6
	7.8	100	4.7±0.9	2.8±1.0	54.7±3.1	26.8±2.3	22.9±2.2	1.3±0.2	1.7±4.6
C	C-10	100	6.8±1.3	3.2±0.1	39.2±1.7	36.3±0.2	20.8±0.3	1.3±0.0	12.4±2.1
	C-30	100	5.8±1.1	2.5±0.4	46.8±4.6	29.1±0.4	17.0±0.8	1.0±0.1	14.4±4.8
	C-50	100	3.8±0.7	2.2±0.4	61.5±2.8	24.6±0.9	14.4±0.9	0.9±0.1	4.6±3.2
	V-10	100	5.8±1.1	3.0±0.2	46.9±5.7	39.7±0.5	22.9±0.4	1.3±0.0	-2.0±5.8
	V-30	100	4.0±0.8	2.4±0.5	59.9±1.6	28.6±0.7	16.8±1.0	1.0±0.1	0.2±2.2
	V-50	100	3.1±0.6	2.0±0.3	66.5±2.3	24.9±0.1	14.8±0.7	0.9±0.1	-1.9±2.4

2