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

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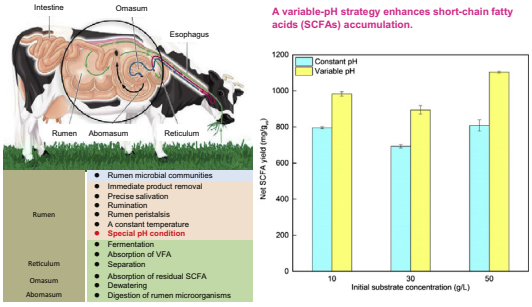
HIGHLIGHTS

- Maize straw was fermented to produce short-chain fatty acids (SCFAs) with rumen microorganisms.
- Higher inoculum ratio shows higher hydrolysis and acidogenesis rate of maize straw fermentation.
- Neutral pH is optimal pH for hydrolysis process of maize straw fermentation.
- pH between 5.8 and 7.8 does not influence acidogenesis process of maize straw fermentation significantly.
- A variable pH condition promotes acid accumulation in maize straw fermentation.

GRAPHICAL ABSTRACT



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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.

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

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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. 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

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).