

Influence of combined sulfachloropyridazine sodium and zinc on enzyme activities and biogas production during anaerobic digestion of swine manure

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ABSTRACT

In order to study the influence of different concentrations of zinc and sulfachloropyridazine sodium (SCPS) on anaerobic digestion (AD) during biogas production, we determined the levels of urease, dehydrogenase activity, and volatile fatty acids (VFAs) in batch tests. The experiments were conducted in small AD devices at a temperature of 37 °C using swine manure and wheat straw as raw materials. Four digestion trials were performed using different zinc and SCPS contents: control digestion with no additives (CK), SCPS at 630 mg kg⁻¹ dry weight (S), SCPS at 630 mg kg⁻¹ with zinc at 500 mg kg⁻¹ dry weight (SL), and SCPS at 630 mg kg⁻¹ with zinc at 5,000 mg kg⁻¹ dry weight (SH). The biogas accumulation under S was 1.7 times that with CK, while SL and SH produced 78% and 35% of that under S, respectively. Correlation analysis showed that the accumulated biogas was significantly negatively correlated ($p < 0.05$) with VFAs, and the urease activity was significantly negatively correlated ($p < 0.01$) with zinc and significantly positively correlated with VFAs ($p < 0.05$). The dehydrogenase activity was strongly correlated ($p < 0.01$) with the biogas accumulated during the AD of swine manure.

Key words | anaerobic digestion, bio-available zinc, combined pollution, dehydrogenase activity, sulfachloropyridazine sodium, urease activity

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INTRODUCTION

Due to the rapid growth of livestock and poultry production, manure represents a severe environmental problem, which may threaten the sustainable development of agriculture in China. Based on a combination of macro-statistics and field survey data, [Qiu *et al.* \(2013\)](#) predicted the future development of the overall emissions and pollution from animal manure in China using the decision support system for Chinese Agriculture Sustainable Development (CHINAGRO), which showed that the total pollution attributable to livestock manure will increase significantly to 298 million t by 2020. Livestock manure has a high organic matter content ([Weiland 2010](#); [Wei *et al.* 2014](#)) and anaerobic digestion (AD) is the most suitable method for its treatment and disposal, where microorganisms produce biogas by bio-degrading waste in this biological process ([Weiland 2010](#); [Jiang *et al.* 2011](#)).

However, in order to maximize economic benefits, promote animal growth, improve the feed efficiency, and treat

diseases, various veterinary antibiotics and some trace heavy metals, such as copper, zinc, and arsenic, are used widely in large farms. Unfortunately, the antibiotics and trace heavy metals incorporated in feed materials cannot be absorbed completely by animals and 40–95% of the total is excreted in dung and urine ([Halling-Sorensen *et al.* 2000](#); [Cang 2004](#); [Jjemba 2006](#)). Thus, the inclusion of mineral elements and antibiotics in feed to enhance animal growth and for therapeutic purposes means that large amounts of antibiotics and heavy metal residues are present in manure ([Ji *et al.* 2012](#)). The effects of antibiotics on AD have been determined in previous studies. For example, [Massé *et al.* \(2000\)](#) showed that the presence of penicillin or tetracycline (550 mg kg⁻¹) in manure slurries reduced methane production by 35% and 25%, respectively. In addition, [Beneragama *et al.* \(2013\)](#) demonstrated that the inclusion of oxytetracycline at a concentration of 30 mg L⁻¹ resulted in a 70.3% reduction in methane production. Some studies

have also addressed the effects of multiple antibiotics. Thus, [Álvarez *et al.* \(2010\)](#) demonstrated the inhibitory effects of oxytetracycline combined with chlortetracycline on AD. [Aydin *et al.* \(2015\)](#) also investigated the combined effects of erythromycin–tetracycline–sulfamethoxazole and sulfamethoxazole–tetracycline antibiotics on the performance of anaerobic batch reactors. Several studies have also investigated the effects of heavy metals on anaerobic fermentation, such as the impact on AD processes, biogas production, and anaerobic ammonium oxidation ([Mudhoo & Kumar 2013](#); [Kimura & Isaka 2014](#)). It has been shown that the toxicity of heavy metals is one of the main causes of instability and failure for anaerobic digesters. However, investigations of the effects of antibiotics combined with heavy metals on the AD performance of livestock manure are limited ([Arikan *et al.* 2006](#); [Altaş 2009](#); [Beneragama *et al.* 2013](#); [Zhe *et al.* 2018](#)), and thus experimental studies are necessary.

Sulfachloropyridazine sodium (SCPS, $C_{10}H_8ClN_4NaO_2S$) is a broad-spectrum sulfonamide and the most commonly used antibiotic in veterinary medicine ([Zhang *et al.* 2015](#); [Wang *et al.* 2017](#)). SCPS is used mainly in livestock and poultry to combat *Escherichia coli* and *Pasteurella* infections, as well as being used widely for disease prevention. In the Haihe River basin in China, SCPS was detected in swine farms at a maximum concentration of $47 \mu\text{g L}^{-1}$, which suggests its potential utility as an indicator of pollution from livestock sources ([Luo *et al.* 2011](#)). However, the effect of SCPS on the performance of AD has not been explored. A study of heavy metal pollution in swine manure in China showed that zinc was present at high concentrations ([Wang & Wei 2013](#)). Hence, in the present study, we tested swine manure as a raw material for AD, where we added SCPS and zinc (as zinc sulfate) to evaluate their effects on AD.

The AD process is complex and the conversion of organic compounds into methane involves processes performed by several microbial groups in four steps, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis ([Town *et al.* 2014](#)). Extracellular enzymes have the main role during the hydrolysis stage, whereas intracellular enzymes are most important in the hydrogen and acid production phases. The products of the hydrolysis and fermentative acidogenesis phases provide the basis for methanogenesis ([Bryant 1979](#)). Thus, variations in the activity levels of intracellular and extracellular enzymes during AD can lead to dynamic changes in the decomposition of organic material, as well as affecting biogas production. We evaluated the activities of urease and dehydrogenases, which are extracellular and intracellular enzymes, respectively.

In this study, we evaluated the effects of adding only SCPS and SCPS combined with zinc during the AD of swine manure. We employed biogas production, enzyme activities (urease and dehydrogenases), and volatile fatty acids (VFAs) as the main evaluation parameters.

MATERIALS AND METHODS

Description of raw materials and experimental design

Detailed description of raw materials, setup, operation and performance of the reactors has been reported in the previous study ([Zhang *et al.* 2017](#)). To summarize, the swine manure (produced by swine fed with leftovers containing no additives) and wheat straw were collected from a small local farm in Yangling, Shaanxi, China, where the pigs did not consume feed containing any antibiotics or heavy metal elements. The wheat straw was crushed and passed through a 2-mm sieve. As described previously, the AD experiments were conducted at 37°C using a constant temperature water bath (DK-600, Shanghai Jing Hong Laboratory Instrument Co. Ltd, China). The digestion reactions were conducted in 4-L digestion tanks with working volumes of 2.8 L. The total solids contents of the AD digesters were all maintained at 8% using deionized water. All of the reactors were gently mixed manually for approximately 1 min per day before measuring the biogas volume. Four digestion trials were performed, i.e., CK: control digestion with no additives; S: SCPS at 630 mg kg^{-1} dry weight (DW); SL: SCPS at 630 mg kg^{-1} with ZnSO_4 solution to obtain a zinc content of 500 mg kg^{-1} DW; and SH: SCPS at 630 mg kg^{-1} with ZnSO_4 at $5,000 \text{ mg kg}^{-1}$ DW. Each treatment was repeated in triplicate. The added concentration of SCPS was based on the hypothesis that 50 mg kg^{-1} (body weight) SCPS was applied once every day and that 25% of the SCPS was released into the environment, with a swine excrement rate of 6.5 kg per day and a moisture content of 85%. The calculation result is $628.21 \text{ mg kg}^{-1}$ (DW). We added $630.00 \text{ mg kg}^{-1}$ (DW) to represent the minimum level of SCPS residues in swine manure. Based on the survey results for zinc contents of swine manure from farms in China ([Dong *et al.* 2008](#); [Peng *et al.* 2010](#)), we selected two concentrations to explore: 500 mg kg^{-1} DW and $5,000 \text{ mg kg}^{-1}$ DW.

Samples were collected in brown bottles on days 0, 7, 16, 31, 46, and 52 for all treatments. For each of the four digesters, the initial mixture (CK) was sampled on day 0. Biogas was collected with 50 mL plastic syringes every

3 days from a gas-collecting bottle (Zhang *et al.* 2017). The biogas was analyzed within 1 day of collection.

Analysis methods

The biogas volume produced by each reactor was recorded at 18:30 every day based on water displacement. The methane contents were measured by gas chromatography using a flame ionization detector (7890A gas chromatograph; Agilent, USA). The pH was determined using a pH meter (Mettler Toledo, Switzerland). The soluble chemical oxygen demand (COD) was determined with an AQ4001 COD analyzer (Termo Orion, USA). The concentrations of VFAs, including acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate, were obtained by gas chromatography (Shimadzu GC2010, Japan) (Tian *et al.* 2015). Zinc was extracted using diethylenetriaminepentaacetic acid (DTPA) and analyzed with a flame atomic absorption spectrometer (Hitachi, Japan). DTPA-extractable Zn was defined as bio-available zinc (bio-Zn) (Roosa *et al.* 2014).

The activities of specific enzymes were determined as follows. One activity unit (U) of urease was defined as the amount of enzyme required to produce 1 mg $\text{NH}_4^+\text{-N}$ per 24 h by urea hydrolysis (Guan 1986). The dehydrogenase activity was determined based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC), as described by Zhu *et al.* (1996) with a slight modification. First, 10 mL of the sample was placed in a 50 mL centrifuge tube, before vortexing for 1 min with glass beads (from the DNA isolation system) to increase the efficiency of cell disruption, and centrifugation at 7,000 rpm for 5 min. The supernatant was discarded. This procedure was repeated twice by adding normal saline to 10 mL and stirring the suspension before measuring the dehydrogenase activity. Next, 2 mL of the liquid was placed in a 15 mL centrifuge tube, before adding 2 mL Tris-HCL (pH = 7.6), 2 mL 0.1 mol L^{-1} glucose, and 2 mL 0.5% TTC. This mixture was incubated at 37 ± 1 °C in a constant temperature box for 4 h. The reaction was terminated by adding 0.1 mL sulfuric acid, followed by extraction with 5 mL methylbenzene, and shaking at setting 7 for 10 min in a vortexer (Vortex Genie, Scientific Industries, Inc.). Next, the mixture was centrifuged at 7,000 rpm for 5 min and the upper organic solvent layer was used for colorimetric analysis.

Statistical analyses

The data obtained in this study comprised the mean values based on three replicates. Significant differences were

detected by analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$. One-way ANOVA and correlation analysis were conducted using SPSS 16.0 for Windows. Changes in the parameters evaluated in this study were tested using SigmaPlot 12.5.

RESULTS AND DISCUSSION

Bio-zinc

It has been pointed out that the determination of total heavy metal content is not enough to indicate its bioavailability, mobility and toxicity (Hsu & Lo 2001; Amir *et al.* 2005). Actually, toxicity of heavy metals are more often dependent on their bioavailability (Nomedá *et al.* 2008; Roosa *et al.* 2014). However, previous studies have shown that the environment can affect the toxicity and biological availability of heavy metals (Bhogal *et al.* 2003). Thus, we measured the bio-Zn contents of the digestate obtained from the AD process and Figure 1 shows the changes in bio-Zn during AD. In SL, the bio-Zn contents of the digestate increased from 55.1 mg kg^{-1} (DW) to 70.8 mg kg^{-1} (DW) during 7 to 46 days, before decreasing to 61.1 mg kg^{-1} (DW) at the end of digestion (52 days). In SH, the bio-Zn contents ranged from 342 mg kg^{-1} (DW) to 377 mg kg^{-1} (DW), but decreased to the lowest level after 31 days. We found that the bio-Zn contents of the digestate comprised 6.80–14.2% of the total zinc, which may have been due to the adsorption of zinc (Demirbas 2008) or its precipitation as a metal sulfide in the anaerobic environment (Prasad & Jha 2010).

Biogas production

The batch reactors were incubated at 37 °C for 52 days. Biogas production started immediately on the first day in all of the digesters. The peak daily biogas production rates were calculated as 1.70, 2.59, 2.04, and 1.97 L day^{-1} after 16, 22, 29, and 28 days of digestion in CK, S, SL, and SH, respectively. The reactors with only SCPS increased the peak daily biogas production rates but delayed the presence time of this phase. Compared with S reactor, the different added zinc reactors (SL and SH) inhibited the biogas production with varying degrees, and also delayed the presence time of peak daily phase. Figure 2 shows the cumulative biogas yields (L) produced with zinc and SCPS added to the reactors. After AD for 52 days, the accumulated biogas levels produced by the CK, S, SL, and SH reactors

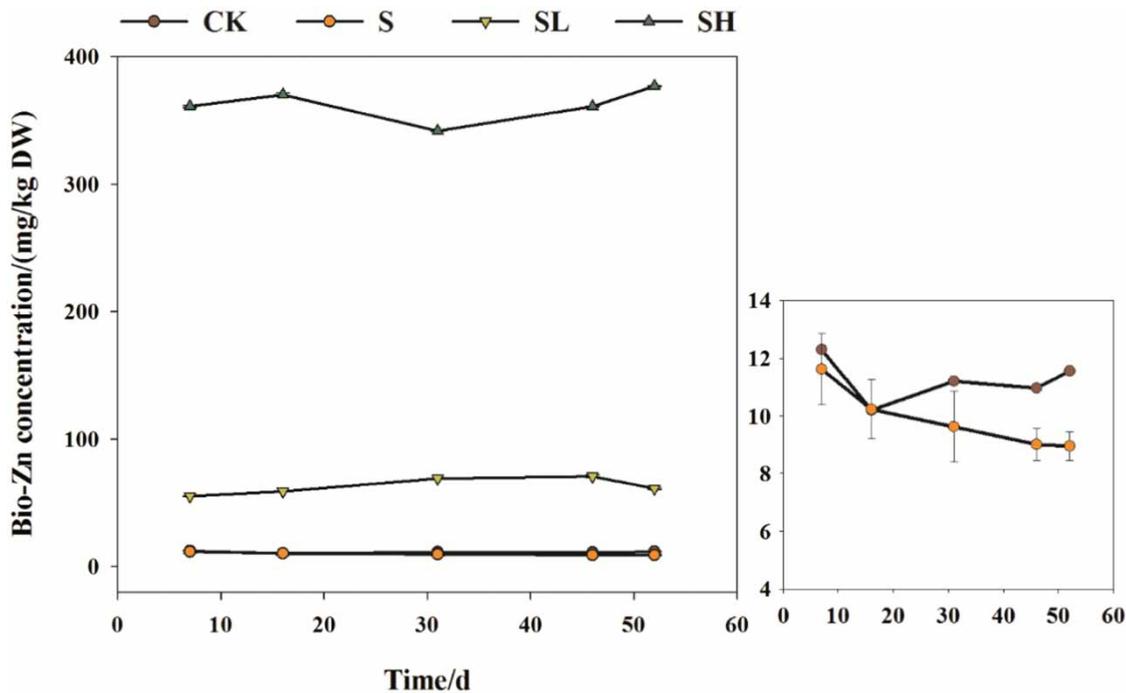


Figure 1 | Bio-available zinc content by different digesters during AD of swine manure.

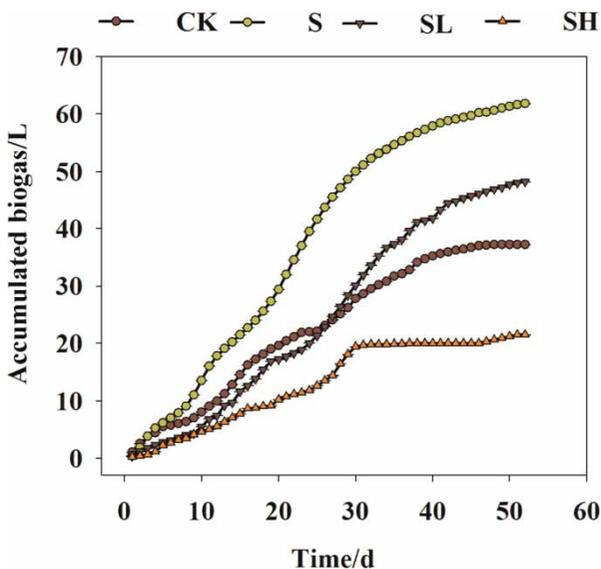


Figure 2 | Accumulated biogas production by the different digesters on a daily basis during AD of swine manure.

were 37.2, 61.8, 48.2, and 21.5 L, respectively. SCPS improved the accumulated biogas (1.7 times), whereas there was a distinct decreased when zinc was added (22% and 65% in SL and SH respectively). Overall, these results demonstrate that the addition of only SCPS changed the AD process although it increased the potential for production of biogas. Furthermore, the combined presence of

SCPS and zinc decreased the biogas production and the degree of inhibition is related to concentration of zinc.

During the AD of swine manure, the biogas production rate each day by the S reactor with added SCPS only was significantly higher than that by the CK reactor. Thus, the reactor with added SCPS produced a large amount of biogas and the accumulated biogas production at 52 days was 1.7 times higher than that with the CK reactor. Our results contradict those obtained in previous studies, which reported reduced biogas production during the AD of swine manure in the presence of the most extensively used antibiotics. For example, *Arikan et al. (2006)* reported that the cumulative biogas production level was 27% lower after the AD of manure obtained from calves medicated with $22 \text{ mg kg}^{-1} \text{ d}^{-1}$ of oxytetracycline for 5 days compared with the AD of manure produced by non-medicated calves. Furthermore, *Aydin et al. (2015)* reported the significant inhibition of biogas production by an antibiotic mixture containing tetracycline, sulfamethoxazole, and erythromycin in AD batch reactors. It should be noted that *Álvarez et al. (2010)* compared their results with previous studies using the same antibiotics and demonstrated some differences in the inhibitory effects according to the operational conditions employed in each study. In the present study, in addition to the different working conditions, the use of SCPS may have been a major explanation for the

improvement in biogas production. On the one hand, SCPS can be adsorbed by organic matter. On the other hand, SCPS compound is rapidly transformed into forms that are less active than itself during the AD process. These may reduce the effect of antibiotic on anaerobic microorganisms (Lallai et al. 2002; Cetecioglu et al. 2013). Additionally, SCPS may have enhanced the activity of resistant bacteria to change the overall microbial community structure (Demoling et al. 2009), thereby improving the capacity for the microbial degradation of organic matter by enhancing the activity of enzymes. Furthermore, Camprubí et al. (1988) have reported that some antibiotics such as chlortetracycline, tylosin and erythromycin did not inhibit methanogenic activity, which may increase the biogas production. Thus, the specific effects of SCPS on activity of bacteria during AD should be investigated in a further study.

The biogas production rates of the SL and SH reactors fluctuated greatly and they were lower than that of the S reactor. The cumulative biogas production rates by the SL and SH reactors were 78% and 35% of that by the S reactor, respectively. Thus, for the SL and SH reactors, the addition of zinc reduced the total biogas production potential during AD. Previous studies that explored the toxicity of zinc in AD systems obtained different results, where 400 mg L⁻¹ zinc had no inhibitory effects on AD and it increased gas production (Zayed & Winter 2000; Li & Fang 2007), whereas Alta (2009) found that only 7.5 mg L⁻¹ zinc had toxic effects. The total zinc concentrations of this study were 125 mg L⁻¹ (SL) and 1,250 mg L⁻¹ (SH). The different results may be attributable to the various material and AD conditions. However, these results confirmed that the concentration of heavy metal was a meaningful factor for the severity of inhibition on AD (Mudhoo & Kumar 2013). Furthermore, Hsu et al. (2001) found that the toxicity of heavy metals such as zinc was attributable to disruption of the structure and function of enzymes, where it combined with the protein molecule thiol group or other groups, or the metal was a component of an auxiliary enzyme. Thus, the toxicity of zinc by inhibiting the activity of enzymes during AD may explain the reduced effectiveness of SCPS in stimulating biogas production and improving the stability of AD. The SH reactor produced no biogas from days 39 to 46 but it began to produce biogas again (326 mL) on day 47, which indicates that the presence of a high level of zinc may have caused the gas production system to become unstable. The high concentration of zinc may have inhibited the growth of microorganisms by affecting the structure and function of enzymes, thereby hindering AD (Chen et al. 2008).

Figure 3 shows the methane contents (%) for the different digesters. The methane contents (%) increased until days 19, 13, 16, and 19 in the CK, S, SL, and SH digesters, respectively, before remaining almost constant subsequently. Thus, the addition of SCPS alone (S) accelerated AD to produce methane at a steady rate. By contrast, when SCPS and zinc were present at the same time in the SL and SH reactors, the methane production rates were stable and did not differ significantly ($p > 0.05$) with S and CK reactors. Therefore, the different concentrations of zinc as well as the presence of SCPS had diverse effects on the biogas yield during the AD of animal manure, but they had little effect on the methane output because all of the reactors entered a stable methane-producing phase.

Enzyme activities

Urease is a hydrolase that attacks linear amide C-N bonds, which can catalyze the transformation of amide compounds into ammonia in a manner that is closely related to nitrogen transformation (Liang et al. 2009). Urease has the ability to decompose protein, fat, and organic nitrogen matter in the liquid phase. Urease is very important for generating ammonia during biogas production but it also plays a major role in the digestion process. The changes in the urease activity levels are shown in Figure 4(a), which indicates that the maximum urease activity occurred on day 7 in all of the digesters. Polysaccharide, protein, fat, and various other macromolecular compounds are decomposed by many

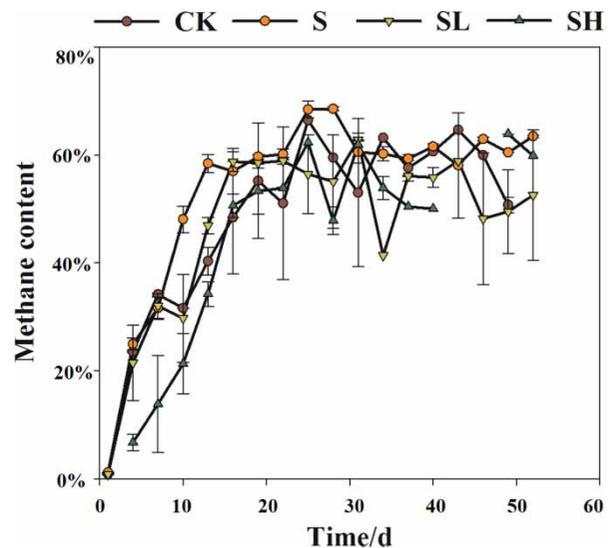


Figure 3 | Methane contents of the biogas produced by different digesters during AD of swine manure. Bars denote standard errors.

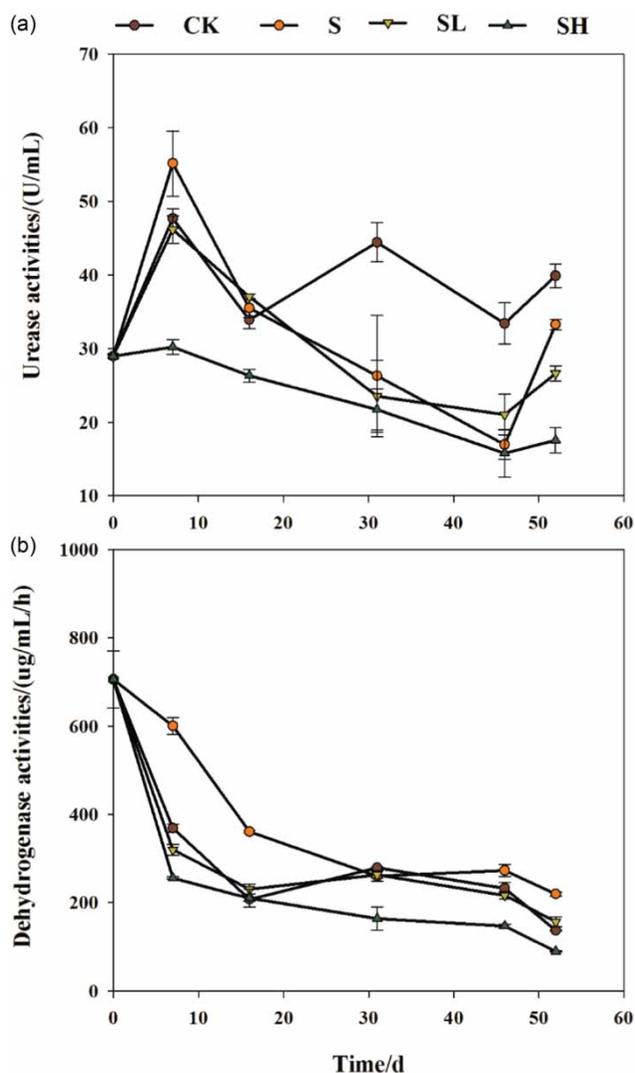


Figure 4 | Urease (a) and dehydrogenase (b) activity levels in different digesters during AD of swine manure. Bars denote standard errors.

extracellular enzymes during the early stage of AD. Thus, our results showed that the urease enzyme activity increased significantly during the early stage of digestion ($p < 0.01$) and then decreased. On day 7, the urease activity was significantly higher in the S reactor ($p < 0.05$) than the CK reactor. However, after 16 days, the urease activity in the S reactor was significantly lower ($p < 0.05$) than that in the CK reactor, where it was above 35.5 U/mL. This result and the biogas production rates suggest that the addition of SCPS accelerated the decomposition of macromolecular compounds by extracellular enzymes. A comparison of the urease activity levels in the S, SL, and SH digesters after 7 days showed that the urease activity was significantly higher in S compared with SL and SH. The urease activity then decreased gradually throughout AD. On day 16, the

urease activity in SL did not differ significantly from that in S ($p > 0.05$), but that in SH was significantly lower than those in both S and SL ($p < 0.05$). After 16 days, due to mainly organic matter decomposition, the urease activity decreased gradually in the S, SL, and SH reactors. However, the activity in SH was always lower than that in SL, which was attributable to the higher concentration of bioavailable zinc in SH than SL. Thus, we suggest that the presence of zinc combined with SCPS may have affected the activity of extracellular enzymes, especially in the SH reactor where the high concentration of zinc influenced the microbial secretion of enzymes (Baldrian *et al.* 2005). This may explain why SL and SH produced biogas at a slower rate during AD.

Dehydrogenases are the major representatives of the oxidoreductase enzyme class (Gu *et al.* 2009) and they play significant roles in the biological oxidation of organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.* 2010). As an intracellular enzyme, dehydrogenase is needed by microorganisms to degrade organic molecules and obtain energy. The changes in the dehydrogenase activity levels are shown in Figure 4(b), which shows that the activity levels decreased gradually during AD of swine manure. The dehydrogenase activity levels in all of the digesters were lower than that in the initial feedstock, which may be explained by a change in the microbial community. The dehydrogenase activity was significantly higher ($p < 0.05$) in the S digester compared with the CK reactor on days 7, 16, and 52, which indicates that the addition of SCPS stimulated the activity of this enzyme. Excluding day 31, the dehydrogenase activities in the SL and SH digesters with added SCPS plus zinc were significantly lower than that in the S digester. Furthermore, the dehydrogenase activity was lower in SH with more added zinc compared with SL. These results show that the toxicity of zinc decreased the activity of dehydrogenase, especially in the SH reactor where the higher amount of added zinc apparently had an inhibitory effect.

Changes in VFA composition

VFAs can be produced via the deamination of the amino acids produced during protein degradation and the breakdown of carbohydrates. During the acidogenesis stage, AD produces various types of VFAs such as formic acid, propionic acid, butyric acid, and isobutyric acid (Zhu 2000), but the main VFA is acetic acid. In this study, we analyzed six types of common VFAs to understand the effects on AD of different concentrations of zinc combined with SCPS. The presence of VFAs throughout the AD processes in all of

the reactors are shown in Figure 5. During the early stage of AD (7 days), the accumulated VFAs (including acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid) were the highest. The total concentrations were 25.3, 17.7, 20.4, and 21.8 g L⁻¹ for CK, S, SL and SH reactors, respectively. These results may be attributed to the hydrolysis and acidogenesis phases of AD (Liu et al. 2015). In all of the reactors, only small amounts of acids accumulated on day 16, and no acids were accumulated on days 31, 46, and 52.

For the six acids evaluated in this study, the contents in the S reactor with added SCPS were 54–95% of those in CK on day 7. The addition of SCPS stimulated the utilization of VFAs, especially propionic acid, which comprised only 54% of that in the CK reactor. Previous studies have shown that propionate is utilized most often by Gram-negative bacteria,

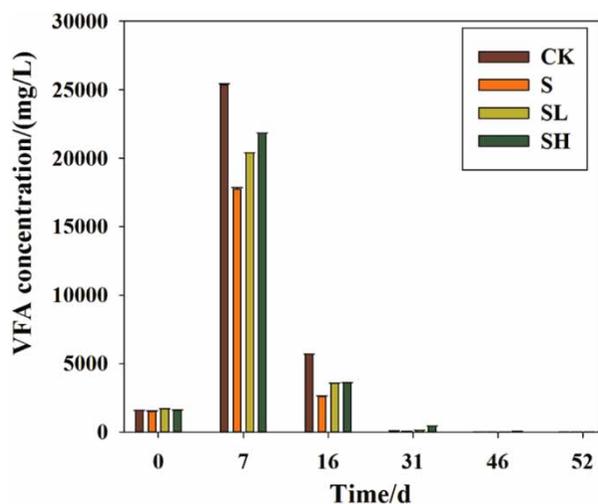


Figure 5 | Distributions of VFAs in all the digesters during the AD of swine manure.

and SCPS is expected to improve the abundance of this microbial group (Aydin et al. 2015). This is consistent with the enhancement of biogas production by SCPS. On day 7 during the early stage of AD, the acetic acid contents were significantly lower in S than SL and SH ($p < 0.05$), which was also the case for butyric acid, isobutyric acid, and valeric acid. There were no significant differences in the propionic acid and isovaleric acid contents in S and SL, but the contents were higher in SH than S. These results demonstrate that the addition of SCPS and zinc can inhibit the effectiveness of VFA utilization. The bio-Zn contents ranged from 341.51 mg kg⁻¹ (DW) to 376.68 mg kg⁻¹ (DW) and they affected all of the acids evaluated in this study, possibly because zinc can inhibit the replication of sensitive strains that have critical roles in the degradation of acid products during AD.

Correlation analysis

In order to characterize the AD systems, we tested the correlations between the levels of bio-Zn, accumulated biogas, urease activity, dehydrogenase activity, and VFAs (i.e. acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid) (Table 1). The accumulated biogas had a significant negative correlation ($p < 0.05$) with the VFAs evaluated in this study during AD, thereby demonstrating that the accumulation of VFAs influenced the generation of biogas. The urease activity had a highly significant negative correlation ($p < 0.01$) with zinc and a significant positive correlation ($p < 0.05$) with VFAs. These results suggest that the toxicity of zinc influences the activity of urease, which may determine the production of VFAs. Urease is a critical enzyme in the acidogenesis phase of

Table 1 | Correlation analysis (Pearson's correlation coefficients)

	Biogas	Bio-Zn	Urease	Dehydrogenase	Acetic	Propionic	Butyric	Isobutyric	Valeric
Bio-Zn	-0.328								
Urease	-0.303	-0.475*							
Dehydrogenase	-0.572**	-0.172	0.257						
Acetic	-0.484*	0.028	0.589**	0.187					
Propionic	-0.478*	-0.042	0.624**	0.091	0.920**				
Butyric	-0.430*	-0.059	0.645**	0.11	0.965**	0.956**			
Isobutyric	-0.500*	0.007	0.589**	0.128	0.953**	0.948**	0.930**		
Valeric	-0.472*	0.117	0.513*	0.045	0.959**	0.910**	0.912**	0.966**	
Isovaleric	-0.524**	-0.008	0.633**	0.182	0.976**	0.956**	0.954**	0.989**	0.964**

*Correlation significant at $p < 0.05$.

**Correlation highly significant at $p < 0.01$.

AD. We found that the dehydrogenase activity had no significant correlation with the bio-Zn and VFAs, but the dehydrogenase activity was strongly correlated ($p < 0.01$) with the amount of accumulated biogas. Thus, the results of this study demonstrate the need to accurately determine the urease and dehydrogenase activity levels during assessments of AD systems.

CONCLUSION

In this study, we showed that the addition of a typical therapeutic level of SCPS to manure stimulated biogas production and accelerated the rate of entry into the stable methane production phase. However, different concentrations of zinc (500 and 5,000 mg kg⁻¹ DW) combined with SCPS inhibited the capacity of AD to produce biogas, as well as delaying the time of entry into the stable phase. In terms of the biogas yield, SCPS was beneficial, but the presence of zinc inhibited the effects of SCPS on AD. Thus, the amount of zinc in livestock manure should be considered before its use in household methane production.

ACKNOWLEDGEMENTS

This study was funded by the National Natural Science Foundation (41671474), the National Natural Science Foundation (41601531) and the 948 Project of Chinese Ministry of Agriculture (2015-Z37). We thank Dr Duncan E. Jackson for critical reading and editing of this manuscript.

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