Enhancing anaerobic digestion of dairy and swine wastewater by adding trace elements: evaluation in batch and continuous experiments

Jing Wang, Maria Westerholm, Wei Qiao IWA, Ahmed Mahdy, Simon M. Wandera, Dongmin Yin, Shaojie Bi, Run Fan and Renjie Dong

ABSTRACT

Trace elements play a critical role for microbial activity in anaerobic digestion (AD) but their effects were probably overestimated in batch tests and should be comparably evaluated in continuous systems. In this study, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} were added in different concentrations to manure wastewater, and the effects were compared in both batch and continuous systems.
The results were used to demonstrate suitable trace element compositions for AD of dairy and swine wastewater, and to compare the outcomes from batch and continuous systems. Fe^{2+} and Zn^{2+} were identified as being the most efficient stimulant of dairy and swine wastewater respectively.
The addition of 5 mg/L Fe^{2+} and 0.4 mg/L Zn^{2+} increased the batch specific methane yield by 62% and 126% for dairy and swine wastewater, respectively. Nevertheless, a lower increment of 2% and 21%, for dairy and swine wastewater was obtained in the 120-day continuously-fed experiments. The 16S rRNA gene sequencing results indicated a relationship between the methanogens population, specific methanogenic activities, propionate, and dissolved hydrogen. Conclusively, the addition of a low dosage of Fe^{2+} and Zn^{2+} is a feasible strategy to enhance the methanogenic metabolism of the AD of dairy and swine wastewater respectively.

Key words | anaerobic digestion, batch and continuous experiment, dairy and swine wastewater, microbial communities and activities, trace elements

Jing Wang
Wei Qiao IWA (corresponding author)
Ahmed Mahdy
Simon M. Wandera
Dongmin Yin
Shaojie Bi
Run Fan
Renjie Dong
College of Engineering,
China Agricultural University,
Beijing 100083,
China
E-mail: *qiaowei@cau.edu.cn*

Jing Wang
Wei Qiao
Simon M. Wandera
Dongmin Yin
Shaojie Bi
Run Fan
Renjie Dong
R&D Center for Efficient Production and Comprehensive Utilization of Biobased Gaseous Fuels, Energy Authority,
National Development and Reform Committee (BG Fuels),
Beijing 100083,
China

Maria Westerholm
Department of Molecular Sciences, Swedish University of Agricultural Sciences,
Uppsala BioCenter,
Box 7025, SE-750 07 Uppsala,
Sweden

Ahmed Mahdy
Department of Agricultural Microbiology, Faculty of Agriculture,
Zagazig University,
44511 Zagazig,
Egypt

INTRODUCTION

In China, around 4 billion tons of animal manure and wastewater is produced annually (Jiang *et al.* 2011). The quantity of cattle and swine manure and wastewater is proportionally the largest at 3.2 billion tons, accounting for approximately 80% of the animal manure generated (Jiang *et al.* 2011). Anaerobic digestion (AD) is considered to be one of the most effective techniques for treating and disposing of manure wastewater in order to mitigate the discharge of chemical oxygen demand (COD) whilst also simultaneously generating clean energy as bio-methane, and producing organic fertilizer as digestate. However, the methane yield from the AD of animal manure is often relatively low and there is a need to find more advanced approaches to increase the efficiency of anaerobic degradation (Qiao

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et al. 2011). The addition of trace elements has been shown to increase methane yield, enhance the degradation efficiency, and alleviate the accumulation of volatile fatty acids (VFAs) in various types of substrate, such as food waste (Westerholm *et al.* 2015), crop residues (Lebuhn *et al.* 2008) and stillage (Gustavsson *et al.* 2011).

Typically, iron, nickel, cobalt, copper and zinc are essential elements for methanogenic enzymes and co-factors. The trace elements were reported to play a key role as co-factors in enzymes for enhancing the methanogenesis (Kida *et al.* 2001). Trace elements can strongly bind to sulfur and thus mitigate the negative effects of sulfide. Recently, the addition of Fe^{2+} and Ni^{2+} was found to alter the methanogenic pathway and subsequently increase the microbial activity under an ammonia-stressed environment (Bi *et al.* 2019). On the other hand, the anaerobic system was normally operated in a continuously fed mode instead of a batch feeding pattern. However, the different effects of the addition of trace elements in batch and continuous AD of manure wastewater as the sole substrate have been infrequently studied. A methane yield enhancement of as much as 55% for chicken manure was reported in a batch experiment by Zhang *et al.* (2012), but a lower enhancement; that is, 34%, was found in a recent study (Bi *et al.* 2019). That may indicate the effects of trace elements were most probably overestimated and should be reevaluated in continuous systems. So far, whether the addition of trace metals can have comparable enhancement effects in batch and continuous anaerobic system is still unclear. On the other hand, although the deficiency of trace elements in the anaerobic system has been widely reported to induce low methane yield and process instability, an overdose could have toxicity effects on the microorganisms (Thanh *et al.* 2016). At the same time, a high dosage of trace elements for enhancing methane production has also been reported; that is, 125 mg/L Zn^{2+} for swine manure (Zhang *et al.* 2017) and 100–500 mg/L Fe^{2+} for chicken manure (Zhang *et al.* 2012). The significant addition of trace elements increases the cost of a biogas plant's operation and may induce excess metals to enter the environment. Consequently, the appropriate dosage used for the trace elements supplements for AD of animal manure and wastewater is a matter that is yet to be answered.

In an anaerobic digestion system, the methanogens utilize acetate or H_2/CO_2 to produce methane. The addition of trace elements thus enhances the activities of methanogens, resulting in an increase in the methane yield. AD is an intricate multi-stage process reliant on the activities of diverse microbial communities for hydrolysis, acidogenesis and methanogenesis. However, there are few studies that have investigated the effects of trace elements on both acetoclastic and hydrogenotrophic methanogenic activities.

This study, therefore, aimed to establish the suitability and appropriate dosage of the trace elements as well as explaining the key role that single trace elements play in a batch experiment. The effects of the trace elements on enhancing the methane yield was further investigated in long term continuously operated digesters, and the methanogenesis activities and the methanogenic communities were analyzed to discover the microbial dynamics with the addition of trace elements.

MATERIALS AND METHODS

Characteristics of wastewater and inoculums

The dairy wastewater was collected from a dairy farm located in Beijing. The sampled wastewater was blended into homogenous slurry in the laboratory by using a blender (Joyoung JYLC012, Jinan) for 5 minutes. The inoculum used in this study was taken from a biogas plant treating dairy manure under mesophilic conditions. The swine wastewater was collected from a swine breeding farm located in Beijing. The inoculum used in the swine wastewater experiment was obtained from a CSTR at this farm. The two digesters were maintained at mesophilic conditions. The diary wastewater had a mean concentration of total solids (TS) and volatile solids (VS) of 44.8 and 35.3 g/L. The TS and VS of the swine wastewater was 8.4 and 4.5 g/L. The inoculum for the dairy and swine wastewater reactor had a TS of 30.8 and 5.8 g/L respectively. The Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} in the dairy wastewater was 0.865, 0.022, 0.041, 0.024 and 0.158 mg/L. Their concentration in swine wastewater was 0.985, 0.109, 0.062, 0.098 and 0.455 mg/L. The characteristics of wastewater and inoculums are provided in Table S1 (supplementary materials).

Batch experiments

Batch anaerobic digestion experiments (30 days) were carried out by conducting biochemical methane potential tests according to a previous study (Wandera *et al.* 2018). The batch experiments were carried out in duplicate at 37 °C using 120 mL serum bottles into which 70 mL of inoculums and 10 mL of dairy wastewater was added. For the swine wastewater experiment, 50 mL of inoculums and 30 mL of swine wastewater were added. The dairy manure and swine wastewater had a VS concentration of 34.5 g/L and 4.5 g/L respectively. In each batch experiment, one of the following trace elements was added to reach different concentrations in the media: Fe^{2+} (5, 10, 20 mg/L), Co^{2+} (0.1, 0.2, 0.4 mg/L), Ni^{2+} (0.1, 0.2, 0.4 mg/L), Cu^{2+} (0.1, 0.2, 0.4 mg/L), Zn^{2+} (0.1, 0.2, 0.4 mg/L). The dosage was chosen based on previously reported values (Zandvoort *et al.* 2006a, 2006b; Qiao *et al.* 2011; Qiang *et al.* 2012; Voelklein *et al.* 2017). Two control bottles filled with inoculums but without substrates and trace elements were incubated in parallel. The gas volume produced from batch bottles was measured using a syringe. The methane content of the biogas was analyzed by using a gas chromatograph (GC-8A, Shimadzu, Japan). The methane production potential was obtained by simulating the gas production using the widely used modified Gompertz model (Wandera *et al.* 2018; Zhao *et al.* 2018) and is provided in the supplementary materials.

Continuously stirred tank reactors

Four glass bottles with a total volume of 2 L (working volume, 1.6 L) were used as the continuously stirred tank reactors (CSTRs). Two of them were used as control. The CSTRs operated in parallel for 120 days and were maintained at 37 ± 1 °C using a water bath (HH-60, ChangzhouGuohua, China). The hydraulic retention time (HRT) of the dairy manure digesters was set at 16 days and the organic loading rate (OLR) was fixed at 2.21 $kgVS/m^3/d$. One reactor received dairy wastewater mixed with Fe^{2+} to reach a final concentration of 5 mg/L. One parallel CSTR treating dairy wastewater without Fe^{2+} addition operated as control. A similar setup was established for the swine wastewater, in which one CSTR received substrate with 0.4 mg/L Zn^{2+} and one control CSTR was fed only substrate. The HRT and OLR of the swine wastewater CSTRs were set at 10 days and 0.45 $kgVS/m^3/day$, respectively. The flow rate of the dairy and swine manure digester were 1.0 and 1.6 L/d respectively. The feeding was conducted manually once per day. The setup of the CSTR system is shown in Figure 1.

Chemical analysis

TS, VS, COD, VFA, NH_4^+-N, and alkalinity were measured according to a previous study (Bi *et al.* 2019). The soluble trace elements in the liquid phase of digestate were analyzed by an inductively coupled plasma-optical emission spectroscopy (Optima 7300DV, USA). The analytical wavelengths (nm) were chosen as follows: Fe (238.204), Co (228.616), Ni (231.604), Cu (327.393), and Zn (206.200). The effluents from the batch serum was centrifuged for 20 minutes at a speed of 10,000 r/min to obtain supernatants. The supernatants were obtained by passing the liquid through membrane filter (pore size, 0.45 μm). The pH of filtrate was adjusted to approximately 2.0 with HNO_3 for 30 minutes. The acidified liquid was further filtered by 0.45 μm membrane filters and then the filtrate was analyzed for concentrations of metal.

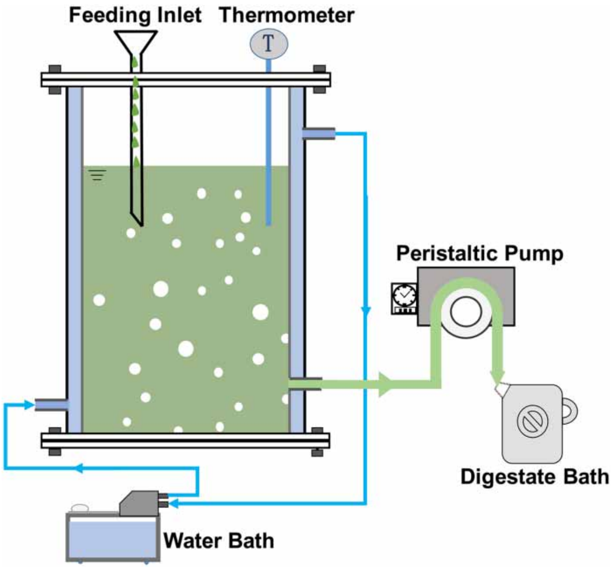

Figure 1 | The setup of the CSTR system.

Acetoclastic methanogenic activity test

The specific methanogenic activity (SMA) of the CSTR were measured by using $NaCH_3COO$ (COD 2,000 mg/L) and $NaCH_3CH_2COO$ (COD 500 mg/L) as the methanogens' substrates. The SMA was obtained by dividing the slope of the linear section of the accumulated methane production rate with the mass of volatile solid weight (Bi *et al.* 2019). The volume of the biogas and methane content was measured at an interval of two hours in the batch experiments.

Hydrogenotrophic methanogenic activity test

The consumption rate of the dissolved hydrogen by hydrogenotrophic methanogens was tested through incubating batch bottles in a water bath measuring 37 ± 1 °C. Two glass bottles (100 mL) were used in the experiment. The first bottle was filled with 70 mL of fresh effluent from the digesters and 70 mL of hydrogen saturated water (37 °C). It was subsequently sealed with air tight cellotape. The second bottle (control) was filled with 70 mL of distilled water and 70 mL of hydrogen saturated water. The consumption rate of the dissolved hydrogen in the two bottles was determined by two H_2-100 microsensors under +1,000 mV connected to an in-situ microsensor (Unisense, Denmark). The hydrogen microsensors were pre-polarized by using distilled water under +1,000 mV for 24 h and then was calibrated by water saturated with hydrogen (Zhao *et al.* 2018). Duplicate experiments were carried out for measuring the hydrogenotrophic methanogenic activities.

The first order kinetic model was used to simulate the consumption rate of dissolved hydrogen.

$$\ln\left(\frac{C_{s_0}}{C_s}\right) = kt \tag{1}$$

where C_{So} is the maximum gas produced (mL); C_s is the maximum gas production minus the accumulated gas (mL); k is rate constant (h^{-1}); t is the time (h).

The analysis of microbial community

Sludge samples were withdrawn from the continuously operating digesters after 120 days of operation and stored at −20 °C until analysis. The CTAB/SDS method was used for total genomic DNA extraction (Chen *et al.* 2010). The polymerase chain reaction (PCR) amplifications were implemented by using the primer 338F/806R for bacterial and 524F/958R for archaeal sequences. The thermal cycling process was performed according to a previous research (Wandera *et al.* 2018). The mixture PCR products were purified by using the Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using the NEB Next® Ultra™ DNA Library PrepKit for Illumina (NEB, USA). The library quality was assessed on the Qubit@ 2.0Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina HiSeq platform, and 250 bp paired-end reads were generated. The paired-end reads from the original DNA fragments were merged using FLASH (Magoc & Salzberg 2011) and was analyzed according to a previous research (Caporaso *et al.* 2011). Finally, the sequences were clustered into operational taxonomic units (OTUs) (at 97% similarity). Typical sequences were chosen for each OTU. Taxonomic classification was performed with 16S rRNA reference (RDP) database. The detailed analysis of microbial community methods were introduced in a previous study (Jiang *et al.* 2019) and added in the supplementary methods.

RESULTS AND DISCUSSION

Effects of trace elements on batch methane production potential

The addition of trace elements enhanced the methane yield from dairy manure waste water in the 30-day batch experiments (Table 1). Fe^{2+} had the most significant effect and

Table 1 | Methane production potential of dairy wastewater in batch experiments with and without the addition of trace elements

		Dairy wastewater				**Swine wastewater**			
	Added trace elements (mg/L)	**P_0 (mL-CH_4/gVS$_{in}$)**	**P_0 increase (%)**	**R_{max} (mL-CH_4/gVS$_{in}$/d)**	**λ(d)**	**P_0 (mL-CH_4/gVS$_{in}$)**	**P_0 increase (%)**	**R_{max} (mL-CH_4/gVS$_{in}$/d)**	**λ(d)**
Control	0	351	/	28	<0.1	1,016	/	70	5.8
Fe^{2+}	5	570	62	31	<0.1	1,058	4	65	5.5
	10	422	20	30	<0.1	1,174	16	87	2.3
	20	401	14	31	<0.1	1,430	41	70	4.7
Co^{2+}	0.1	430	22	32	<0.1	1,032	2	77	2.6
	0.2	413	18	32	<0.1	1,029	1	75	2.1
	0.4	404	15	31	<0.1	901	11	69	2.5
Ni^{2+}	0.1	385	10	31	<0.1	1,096	8	80	2.5
	0.2	411	17	30	<0.1	1,075	6	74	2.4
	0.4	438	25	33	<0.1	931	−8	40	2.3
Cu^{2+}	0.1	383	9	28	<0.1	978	−4	70	2
	0.2	378	8	31	<0.1	1,030	1	74	2
	0.4	338	−4	27	<0.1	1,414	39	100	3
Zn^{2+}	0.1	364	4	30	<0.1	1,300	28	92	2.6
	0.2	381	9	30	<0.1	1,782	75	116	3.1
	0.4	458	31	34	<0.1	2,300	126	141	4.3

the addition of 5, 10 and 20 mg/L increased the methane yield by 62%, 20% and 14%, respectively (i.e. from 351 to 570, 422 and 401 mL/g-VS_{in}). For the other metals, the highest increase in the methane yield was 31% for 0.4 mg/L Zn^{2+}, 25% for 0.4 mg/L Ni^{2+}, 23% for 01 mg/L Co^{2+}, and 9% for 0.1 mg/L Cu^{2+}. It was supposed that the addition of trace elements enhances the binding with sulfur and, thus, has a positive effect on the AD process. Several other studies have shown positive effects by the addition of Fe salts to manure containing materials (Zhang *et al.* 2016). In the current study, the results indicated that the Fe^{2+} played a critical role in the rise of the methane yield in dairy wastewater. At the same time, the maximum methane production rate (R_{max}) also slightly increased with the addition of each metal (Table 1).

For swine wastewater, a higher methane yield (1,016 mL/gVS_{in}) than that from dairy manure was obtained in the batch experiments (Table 1). At the same time, a significant increment in the methane yield was observed when the elements were added. For example, the addition of 0.2 and 0.4 mg/L Zn^{2+} increased the methane yield by 75% and 126%, respectively. The present study thus shows that even a lower dosage of 0.2–0.4 mg/L Zn^{2+} has comparable and positive effects on the methane yield increment. A significant increase in the methane production rate was also observed with the addition of Zn^{2+}; that is, from 70 to 141 ml/gVS/d (0.4 mg/L Zn^{2+}). The addition of Co^{2+} and Ni^{2+} did not significantly increase the methane yield from swine manure. The highest positive effect on methane yield was obtained from the addition of 0.4 mg/L Cu^{2+} (+39%) and 20 mg/L Fe^{2+} (+41%) in Table 1. This result indicates that both Fe^{2+} and Zn^{2+} have a critical role in the enhancement of the methane yield from dairy and swine wastewater, and that even a lower dosage than that used in previously reported research (Zhang *et al.* 2014, 2016) can play a positive role.

Effects of trace elements on long term CSTR reactors

The batch experiments results indicated that the Fe^{2+} (5 mg/L) had the most significant effect on dairy wastewater, and Zn^{2+} addition was most beneficial in swine wastewater. Consequently, this was further investigated in continuously operating systems. As shown in Figure 2(a), Fe^{2+} addition in the dairy wastewater reactor increased the methane yields by 12% (84 mL/g-VS_{in}) compared with the control (75 mL/g-VS_{in}) with a $P = 0.033$. At the same time, the concentration of VFAs were lower in dairy wastewater after the addition of Fe^{2+} compared to the control digester with a $P < 0.05$. The VFA concentration was shown in Figure 2(c). From the 43th day to the end of the experiment, the acetic acids in the Fe^{2+} added digester were significantly ($P < 0.05$) lower than in the control one. However, compared to the results obtained in the batch experiments, the enhancement of the methane yield in the continuous experiment with Fe^{2+} was much lower.

The summary of the long term continuously-fed reactors performance was provided in Table 2. The swine wastewater continuous digester supplemented with 0.4 mg/L Zn^{2+} had a 21% higher methane production compared to the control (192 in control and 238 mL/g-VS_{in} in Zn^{2+} supplemented reactor). The increment in the continuous experiment was also much lower than that obtained in the batch experiment. A longer time is therefore required to obtain high methane production even with the addition of trace metals. The total concentration of VFAs in the swine wastewater digesters was 192 mg/L without Zn^{2+} and 66 mg/L with Zn^{2+}. The period between the 85th and 122nd day indicated a stable but low VFA concentration, i.e. 53 mg/L (without Zn^{2+}) and 48 mg/L (with Zn^{2+}). As previously reported, the VFA reduced from 2,100 mg/L to 400 mg/L when trace elements (Fe, Co, Ni, Se, and W) were added to the mixture of manure and industrial waste (Qiao *et al.* 2011), suggesting that the methanogenic activity in the swine wastewater digester may be enhanced by adding the trace elements. The VFA concentration in swine digesters was shown in Figure 2(e) and 2(f).

In this study, the continuous digester treating dairy and swine wastewater was operated for 120 days with HRTs of 8 and 12 for the digesters. The enhanced performance obtained from the long term experiment was therefore representative. However, by comparing the batch experiments, the significantly enhanced methane production through the addition of trace metals did not happen in the long term experiment. From this point of view, the evaluation of the effects of trace metals on methane production should be investigated by operating long term continuous digesters to obtain more useful and reliable results.

It was reported that in a neutral pH environment, approximately 50% of the hydrogen sulfide was available as bio-sulfide ion HS^- to potentially precipitate trace elements (Voelklein *et al.* 2017). The precipitation of FeS and the co-precipitation of S with Co, Ni, Zn and Cu in an anaerobic system would affect the uptake of the metals by microorganisms. The Fe was more easily precipitated to FeS and this thus minimized the formation of H_2S (Wei *et al.* 2018). In the current study, the addition of Fe was

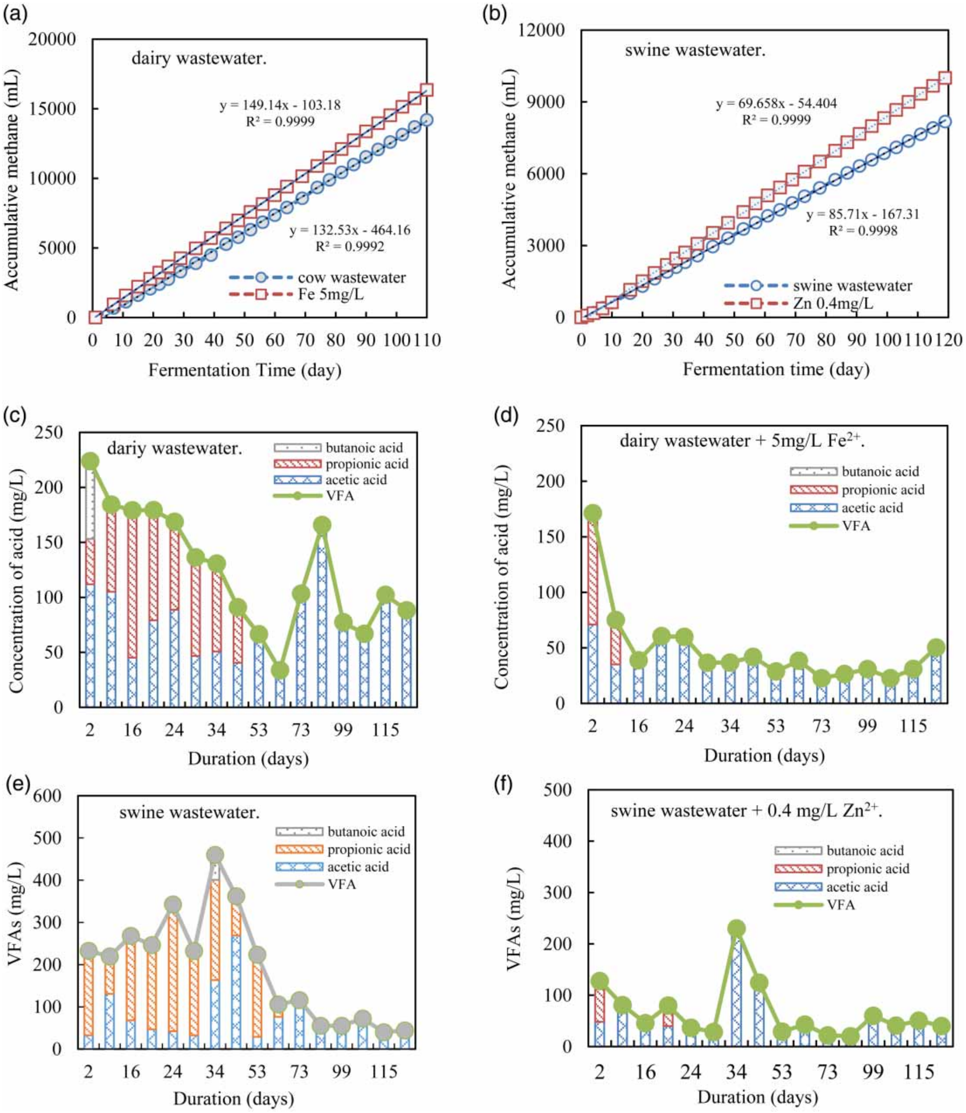

Figure 2 | Methane yield and VFA variations in the digesters with and without the addition of metals.

also higher than for other elements. In light of the observations in the current study, the different roles of Fe^{2+} and Zn^{2+} on dairy and swine wastewater still need further investigation. On the other hand, the effects of trace elements addition in continuous AD was much lower than that obtained in batch AD. The may indicate the evaluation of trace elements effects using batch experiment procedure may overestimated the effects.

Effects of trace elements on microbial communities

The analysis of the microbial community showed that the Clostridia and Bacteroidia were the dominant bacterial classes in the continuously operating digesters after 120 days of operation (Table 3). In the swine wastewater digester, the addition of 0.4 mg/L Zn^{2+} slightly decreased the class Clostridia from 43.8% to 39.2%. At class level, the methanogenic communities were clearly dominated by Methanomicrobia, which represented 88–92% of the total archaeal community in both digesters. The addition of the metals only had minor effects on the methanogens population. Compared to the cow wastewater (2.8–3.2%) reactors, the *Methanobacteria* in swine wastewater (with and without Zn^{2+} addition) was significantly higher, i.e. 11–12%. In cow wastewater digesters, the methylotrophic methanogen Thermoplasmata (Poulsen

Table 2 | Summary of long term digester performance

Parameters	Units	Dairy wastewater	Dairy wastewater + 5 mg/L Fe^{2+}	Swine wastewater	Swine wastewater + 0.4 mg/L Zn^{2+}
HRT	days	16	16	10	10
OLR	g-VS/(L·d)	2.2	2.2	0.45	0.45
pH	/	7.96 ± 0.20	7.98 ± 0.20	7.91 ± 0.18	7.86 ± 0.20
Gas production	mL/(L·d)	203 ± 20	222 ± 7	102 ± 4	127 ± 3
CH_4	%	64.7 ± 5.4	67.6 ± 2.5	67.7 ± 2.5	67.1 ± 2.4
CO_2	%	35.1 ± 4.4	31.6 ± 3.3	33 ± 0.03	32.1 ± 3.8
CH_4 yield	mL/g-VS	74 ± 9	85 ± 3	192 ± 10	238 ± 9
Acetate	mg/L	79 ± 4	39 ± 14	80 ± 3	59 ± 5
Propionate	mg/L	94 ± 7	0	109 ± 7	22 ± 7
Butyrate	mg/L	0	0	0	0
VFAs	mg/L	125 ± 4	48 ± 6	192 ± 8	66 ± 5

Table 3 | Summary of bacterial and archaeal community under class level, %

		Cow wastewater	Cow wastewater (+ 5 mg/L Fe)	Swine wastewater	Swine wastewater (+ 0.4 mg/L Zn)
C_Bacterial	Clostridia	35.2	29.5	43.8	39.2
	Bacteroidia	28.1	31.9	14.9	15.2
	Synergistia	1.8	4.1	0.7	1.3
	Spirochaetes	3.1	4.5	3.5	4.4
	Gammaproteobacteria	5.6	2.8	9.5	12.3
	Fibrobacteria	3.6	2.9		
	Erysipelotrichia	4.6	2.9	1.3	1.0
	Epsilonproteobacteria	3.1	2.8	13.2	16.3
	Deltaproteobacteria	2.3	2.1	1.5	1.3
	Betaproteobacteria	1.7	1.3	4.7	3.3
	Bacteroidetes	2.4	2.2	/	/
	Anaerolineae	2.6	4.8	/	/
	Cloacimonetes	/	/	1.8	1.1
	Others	5.9	8.1	5.1	4.5
C_Archaeal	Methanomicrobia	91.6	91.7	87.9	88.3
	Methanobacteria	3.2	2.8	11.7	11.1
	Thermoplasmata	5.2	5.5	/	/
	Others	/	/	0.4	0.6

Note: the 'others' were less than <1%.

et al. 2013) was also found at 5.2–5.5%; however, in the swine wastewater digesters, the Thermoplasmata was less than 1% (Table 3).

The analysis of the methanogenic communities showed different structures at genus level in diary and swine manure digesters, whereas the addition of Fe^{2+} or Zn^{2+} had a minor effect on the community composition (Figure 3). In dairy wastewater digesters, the obligate acetoclastic methanogen *Methanosaeta* represented 35–40% of the total archaeal community both without and with Fe^{2+} (Figure 3(a)). Similarly, the relative abundance of *Methanosarcina* slightly decreased from 48% to 46% with Fe^{2+} addition. *Methanosaeta* has been shown to use acetate at a low concentration with a minimum threshold of acetate at 7–70 μmol/L, while *Methanosarcina* required higher concentrations of acetate in the range of 200–1,200 μmol/L (Jetten *et al.* 1992). In the current study, the slight decrease in acetic acid concentration from 91 to 31 mg/L and the enhanced methane yield in the digester with the addition of Fe^{2+} indicate that this addition

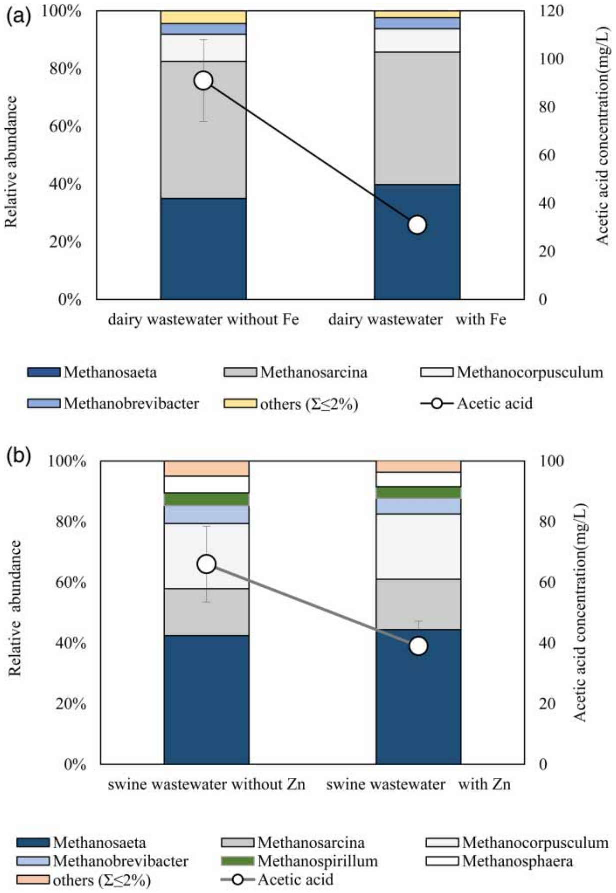

Figure 3 | Methanogenic communities and acetic acid in digesters with and without the addition of metals. (a) Dairy wastewater digester; (b) swine wastewater digester.

of metals may improve the growth and activities of acetoclastic methanogens.

In the swine wastewater digesters, *Methanosaeta* was also the dominant methanogen at a total relative abundance of 42–44%, both with and without the addition of Zn^{2+}. The relative abundance of hydrogenotrophic methanogens (including *Methanocorpusculum*, *Methanobrevibacter*, *Methanoculleus*, *Methanospirillum*, and *Methanosphaera*) was of a similar number of around 37% in the swine wastewater digester, irrespective of the Zn^{2+} addition. *Methanocorpusculum* showed a high percentage in the swine wastewater digester with and without Zn^{2+} addition, and the addition of Zn^{2+} slightly increased from 15.5% to 16.6%. *Methanobrevibacter* accounted for 5.9% and 5.2% for the swine wastewater digester with and without Zn^{2+} addition. *Methanocorpusculum* and *Methanobrevibacter* may have played an important role in the anaerobic digestion of swine wastewater, since those methanogens were previously found in swine waste storage pits (Whitehead & Cotta 1999); however, in the dairy wastewater digester, those two methanogens were not observed.

Effects of trace elements on methanogenic activities

The specific methanogenic activity (SMA) of the dairy wastewater digester showed a significantly increased methane production rate from 0.245 to 0.367 mL-CH_4/gVS/h from acetate when Fe^{2+} was added to the digester (Figure 4). The significant increase in SMA is in line with previous studies showing a 43.1% increase in SMA with trace elements supplementation (iron, nickel, cobalt, and zinc) in an anaerobic membrane bioreactor treating food processing wastewater (Yu *et al.* 2016) and a 74.8% increase with the addition of Co^{2+} (5 μmol/L) (Zandvoort *et al.* 2006a, 2006b). The propionic acid was a major intermediate and important metabolite in the anaerobic process. In the current study, the positive effects of trace elements on propionic acid degradation were also observed. The addition of Fe^{2+} in the digester treating dairy wastewater significantly enhanced the SMA of propionic acid from 0.0469 to 0.0642 mL-CH_4/gVS/h (Figure 4). A high SMA of propionic acid from 0.6875 to 1.0395 mL-CH_4/gVS/h was found in the swine wastewater digesters, with Zn^{2+} addition increasing it significantly by 51.2%.

During the anaerobic digestion process, dissolved hydrogen was an important precursor for methane formation. In the current study, the hydrogenotrophic methanogenesis activity was investigated by testing the dissolved hydrogen concentration in batch experiments. A higher consumption rate ($P = 0.0455$) was found in the dairy wastewater feeding digester with Fe^{2+} addition (Figure 5(a) and 5(b)). The hydrogen consumption rate of dissolved hydrogen increased from 0.1155 h^{-1} in the controlled dairy wastewater digester to 0.1325 h^{-1} in the Fe^{2+} supplemented digester, indicating that Fe^{2+} addition induced the development of archaeal communities with higher activity. Investigation of swine wastewater digesters indicated the SMA of acetic acid was 9.024 and 5.394 mL-CH_4/gVS_{in}/h with and without Zn^{2+} supplement. An increment of SMA by 67.3% was obtained through the addition of 0.4 mg/L Zn^{2+}. However, the dissolved hydrogen consumption rate of swine wastewater was not significantly increased with a P value of 0.11 when Zn^{2+} was added (Figure 5(c) and 5(d)). As the hydrogenotrophic methanogens in swine wastewater with and without metals addition were higher than those in dairy wastewater, it was therefore supposed that the swine wastewater already had higher capacities for hydrogen consumption. Consequently, the addition of Zn^{2+} did not

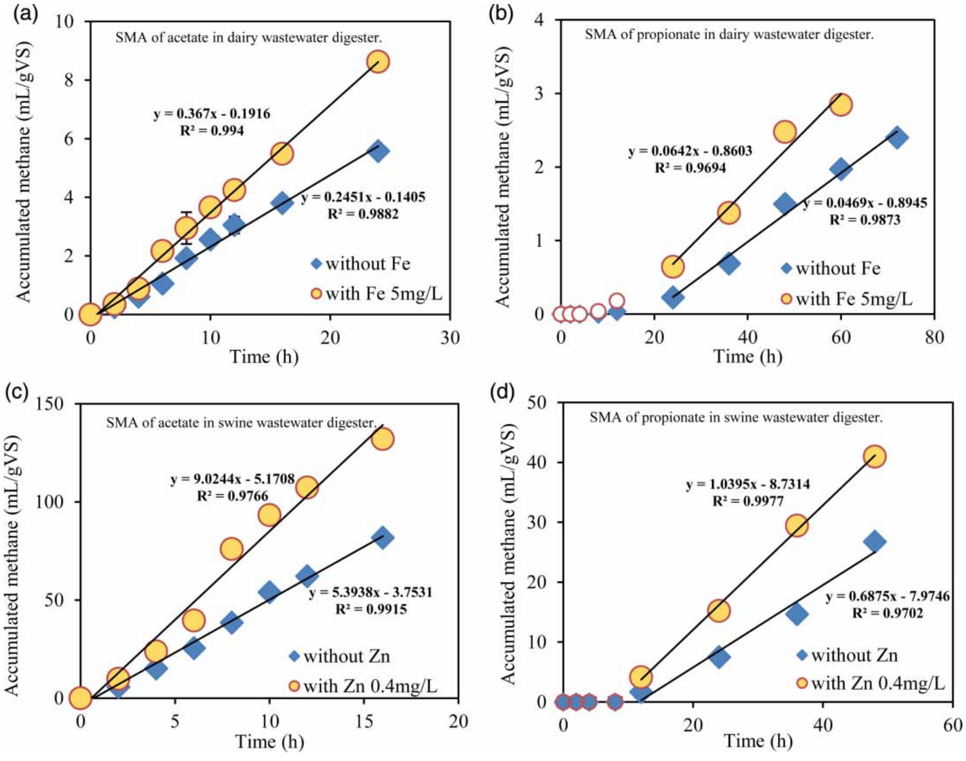

Figure 4 | Specific methanogenic activity of acetate and propionate in dairy and swine wastewater digesters.

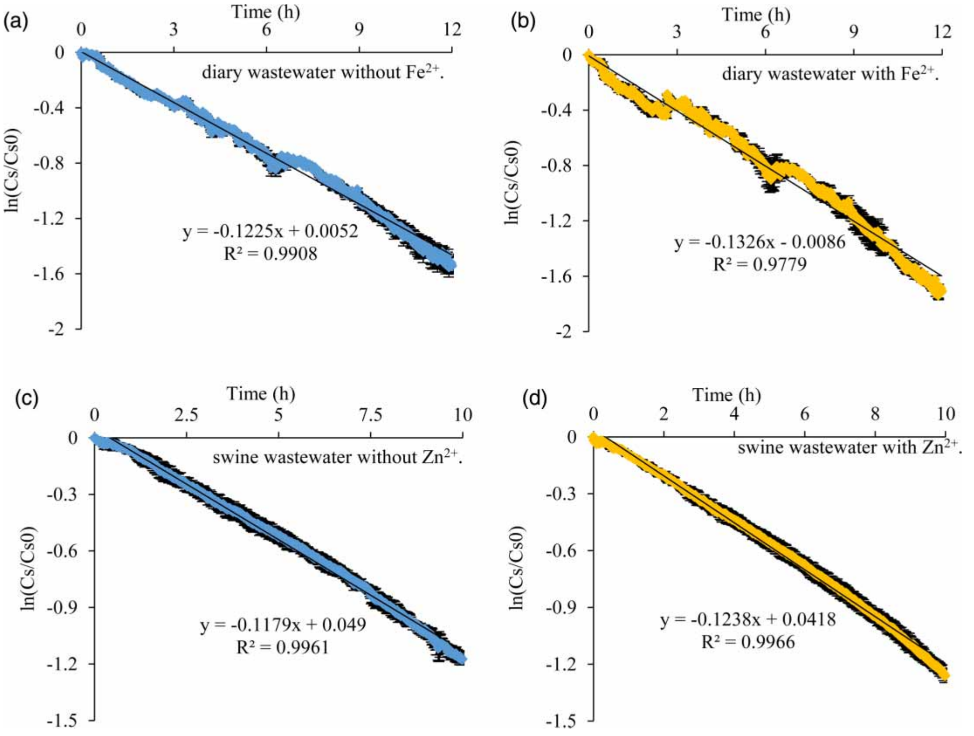

Figure 5 | Hydrogen consumption rate of dairy and swine wastewater digesters.

significantly increase the hydrogen consumption rate in swine wastewater. The results showed that the increment in the methane production yield when trace elements were added in the dairy and swine manure wastewater anaerobic digesters could be induced by the improved methanogenic activities, which also enhanced the conversion of both acetate and propionate and lowered the level of dissolved hydrogen.

CONCLUSION

The effects of each metal on enhancing the methane yield of dairy and swine wastewater were obtained through the batch experiments. The key roles of Fe^{2+} and Zn^{2+} were identified in the anaerobic digestion of dairy and swine wastewater. The methane yield in the long-term continuous experiment was also increased by adding a low concentration of metals but the degree was much lower than those obtained in the batch tests. The addition of Fe^{2+} and Zn^{2+} also induced the changes in the methanogen populations, which may explain the increased consumption rate of the dissolved hydrogen, and the specific methanogenic activities.

Supplementary data for this work can be found in the electronic version of this paper (Figure S1, methane production potential of dairy wastewater with and without the addition of metals; Figure S2, methane production potential of swine wastewater with and without the addition of metals; Figure S3, bacterial community at day 120 of operation with and without the addition of elements; Table S1, Characteristics of inoculum and dairy and swine wastewater used in batch and continuous experiments; Table S2, Summary of bacterial and archaeal community under class level, %; Equation S1, modified Gompertz model; Method S1, the analysis of microbial community).

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SUPPLEMENTARY MATERIAL

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