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Metabolic performance of anaerobic digestion of chicken manure under wet, high solid, and dry conditions

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ABSTRACT

The anaerobic digestion (AD) of chicken manure as a solo substrate has been challenging due to the ammonium inhibition effects when adopting a high organic loading rate (OLR). In this study, through increasing both the total solid in the feeding materials from 5% to 20%, and the OLR from 1.7 to 7.1 g-volatile solids (VS)/(L·d), the AD of chicken manure under wet, high solid, and dry conditions, with a fixed hydraulic retention time of 20 days, was investigated. The results obtained indicated that the wet AD system could achieve a methane yield of 0.28 L/g-VS and a low volatile fatty acid level. However, the process deteriorated under dry conditions, and methane formed mainly through acetate oxidation and methanogenesis. *Methanosarcina* and *Methanoplasma* were found to be more tolerant But, whether the dry AD of chicken manure can survive an ammonia-stressed environment when the OLR is lowered, still needs investigation.

1. Introduction

With greater awareness of climate change and increased levels of environmental protection, it is imperative to increase the use of renewable resources and thereby reduce dependency on fossil energy. Methane production through anaerobic digestion (AD) has the ability to substantially contribute towards the transition to a more sustainable society. With the benefits of a high methane yield, the AD of nitrogen-rich materials, such as chicken manure, is appealing (Chen et al., 2017; Fuchs et al., 2018). In China, about 155 million tons of chicken manure are produced annually (Bi et al., 2019a) and this has the potential to produce 3×10^{14} KJ energy (Wandera et al., 2018). Normally, the AD of chicken manure operated under a wet system by feeding the digester with a total solid content of around 6%-10% (Fuchs et al., 2018; Wang et al., 2015). A large amount of water is thus needed to dilute the raw chicken manure, which has a total solids (TS) content of at least more than 25% (Li et al., 2017; Nie et al., 2015). The consequent increased financial costs of biogas plant construction, and volume increased digestate treatment, make the AD of chicken manure less competitive.

A high solid (feeding TS ≥ 10%) and dry AD (feeding TS ≥ 20%) system have been investigated to increase the cost-effectiveness of an anaerobic process to avoid dilution of the feedstock. However, previous studies have reported that an elevated ammonia level may exert a higher risk on the activities of anaerobic microorganisms in a high solid or/and dry AD system (Abouelenien et al., 2009; Moestedt et al., 2016; Westerholm et al., 2018). Consequently, the disturbance of the process might lead to a decrease in the methane yield. Chicken manure contains high content protein and uric acid which will release high ammonium-N in an AD system, and it has been reported that the AD of chicken manure is inhibited under total ammonia–nitrogen (TAN) concentrations of 4–6 g/L (Niu et al., 2015; Bayrakdar et al., 2017). Long-term acclimation is considered to be a practical way to enhance microbial tolerance to a stressed environment (Moestedt et al., 2016; Abouelenien et al., 2009; Nie et al., 2015).Therefore, the performance of the AD of chicken manure through long term acclimation has been previously investigated under both a fixed organic loading rate (OLR) and ammonia level (Molaey et al., 2018), and under step-increased ammonia levels by artificially adding minimal ammonium-N (Niu et al., 2013). However, the OLR, which is an important influencing factor, and its synergy effects with high ammonia on AD performance, has rarely been studied. So far, it is still unclear whether the high solid and dry AD of chicken manure can endure the high ammonia levels, created as a result

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of long term acclimation through the steep increase in the OLR and ammonia stress.

Methane is produced through acetate cleavage or syntrophic acetate oxidation coupled with the hydrogenotrophic methanogenesis (SAO-HM) pathway. Fundamentally, the microbial community determines the metabolic pathway and the gas production, and the high sensitivity of acetoclastic methanogens to ammonia stress induce the dynamics of the methanogenic pathway (Niu et al., 2015; Moestedt et al., 2016). It has been reported that methane formation via SAO-HM rose from 9% to 23% to 68%-75% as the TAN increased from 0.2 to 1.6 g/L to 3.6–6.1 g/L (Mulat et al., 2016). In addition to this, an increased OLR has also been found to induce the increase in the proportion of methane formation via SAO-HM (Li et al., 2016). However, the dominance of the SAO-HM pathway has normally only been observed when the volatile fatty acid (VFA) accumulation occurs (Westerholm et al., 2019; Yin et al., 2018). Consequently, the question of whether the SAO-HM can support a highly efficient AD of nitrogen rich materials still needs to be answered.

This study therefore aimed to illustrate the performance of the AD of chicken manure under wet, high solid, and dry systems. The biogas production, microbial community, and the methanogenic pathway were investigated through a 560-day consecutive AD experiment by step-increasing the TS in feeding from 5% to 20%. The mechanism behind this was used to explain the correlation between the methane production, microbial community, and the process parameters.

2. Materials and methods

2.1. Experimental set-up and operations

Mesophilic (37 °C) AD of chicken manure was carried out in a continuous stirring tank reactor (CSTR). The digester had a total volume of 16L with a working volume of 12 L, was equipped with a paddle type agitator (100 rpm), and had a water jacket with warm water circulation. The digester was manually fed and effluents were withdrawn manually every day. The biogas was collected by a gas bag and the volume was then measured by a wet gas flow meter (LML-1, Jinzhiye Co., LTD, China). The chicken manure used as substrate in the present study was collected from a caged laying hen farm in Beijing, China. The raw chicken manure had a TS of around 43% and a volatile solids (VS) of around 29%. After collection, the manure was diluted with tap water for wet AD with a TS of 5%, 7.5% and 10%, 15% for high solid AD, and 20% for dry AD. The diluted manure was distributed into 500 mL vials and then stored at 4 °C until use.

The inoculum was taken from a full-scale digester treating pig manure at ~ 37 °C. The inoculum had a 2.1% TS and a 1.1% VS. The HRT was set at 20 days, i.e. daily feeding of 600 mL feedstock. The duration of each phase was 93, 87, 90, 193 and 97 days, respectively (Fig. 1). The gas production, pH, and biogas composition were measured daily. VFA and TAN were measured every 6 days. The interaction among those parameters was performed using the Pearson Correlation Analysis with Microsoft Excel 2016 software.

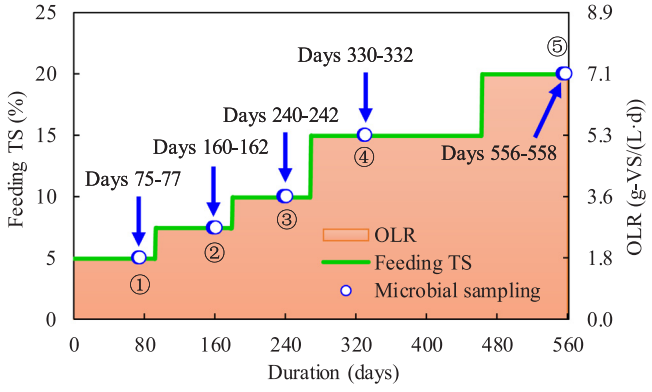

Fig. 1. Total solid (TS) content in feedstock, organic loading rate (OLR), and duration of each setting in anaerobic digester operation.

2.2. Chemical analysis

TS, VS, volatile suspended solids (VSS), and TAN were analyzed according to JSWA (1997). The VFA concentration, methane concentration, and pH were determined as previously described (Bi et al., 2019a). Isotopic analysis of CH_4 and CO_2 were performed via Isotope Ratio Mass Spectrometer (IsoPrime 100, IsoPrime limited, UK) equipped with a cryo-focusing unit (Trace Gas Preconcentrator, IsoPrime limited, UK) with (greater than0.3%) analytical precise.

2.3. The measurement of methanogenic activity

Specific methanogenic activity (SMA) tests were conducted to investigate the methane production ability of methanogens. Two parallel samples were taken for a microbial activity test at each OLR stage. Inoculum for the batch assays were taken from the digester on days 75, 160, 240, 330 and 556. Four 120 mL glass bottles were used for each phase (2 bottles with 4 g-COD/L of sodium acetate and 2 bottles as control). Apart from sodium acetate, inoculum (10 mL) and sterile basic anaerobic medium (90 mL) were added to the bottles. The chemical composition of the medium may be found elsewhere (Li et al., 2015). The bottles were tightly sealed after 2 min of flushing with nitrogen gas in order to form anaerobic conditions. To mimic similar conditions with the digester at each phase, the TAN of the sterile medium was adjusted to 2.5, 5.0, 6.5, 6.9, and 8.5 g/L by using ammonium chloride and the pH was adjusted to 8.2, 8.4, 8, 7.8, and 7.7 by using sodium hydroxide. All bottles were placed at 37 °C until biogas production ceased. SMA was measured according to the following equation (1).

$$\mathrm{SMA} = \frac{1}{\mathrm{VSS}\cdot \mathrm{V_R}\cdot f}\cdot\frac{dV_{\mathrm{CH_4}}}{dt} \quad (1)$$

where $V_{\mathrm{CH_4}}$ refers to the cumulative methane production after calculation on standard temperature and pressure (273.15 K, 100 kPa), mL; $\mathrm{V_R}$ stands for the volume of inoculum, L; f is the theoretical COD conversion to methane (350 mL-CH_4/g-COD) under standard temperature and pressure (273.15 K, 100 kPa); VSS refers to volatile suspended solids of inoculums, 0.89, 1.54, 3.09, 3.99, and 5.01 g-VSS/L for OLR of 1.8, 2.7, 3.6, 5.3 and 7.1 L/(L·d) stages; t is the number of days since inoculation.

2.4. Methanogenic pathway analysis with labeled acetate

The acetate degradation pathway was analyzed with labeled acetate (2-^{13}C). Inoculum for analyses were taken from the continuous digester on days 75, 160, 240 and 330. Bottles containing labelled acetate and inoculum (acetate/inoculum ratio of 20/80) were sparged with nitrogen gas and sealed. The initial acetate concentrations were set at 2, 2.4, 7 and 14 g-COD/L, based on the digester performance at each phase. All experiments were conducted in duplicates. In addition, blank bottles (without acetate addition) were performed for each OLR level. The batch bottles were kept at 37 °C, and the isotopic analyses ($\delta^{13}CH_4$ and $\delta^{13}CO_2$) were applied to the biogas produced. The ratios of methane production from labeled and unlabeled carbon) were determined using the following equations (2)–(4) (Yin et al., 2018):

$$\delta^{13}C = 1000 \times [(R_{sample}/R_{stardard}) - 1] \quad (2)$$

$$f_{mc} = (\delta_{\mathrm{CH_4}} - \delta_{ma})/(\delta_{mc} - \delta_{ma}) \quad (3)$$

$$\delta_{mc} = \frac{\delta_{\mathrm{CO_2}} + 10^3 - \alpha_{mc} \times 10^3}{\alpha_{mc}} \quad (4)$$

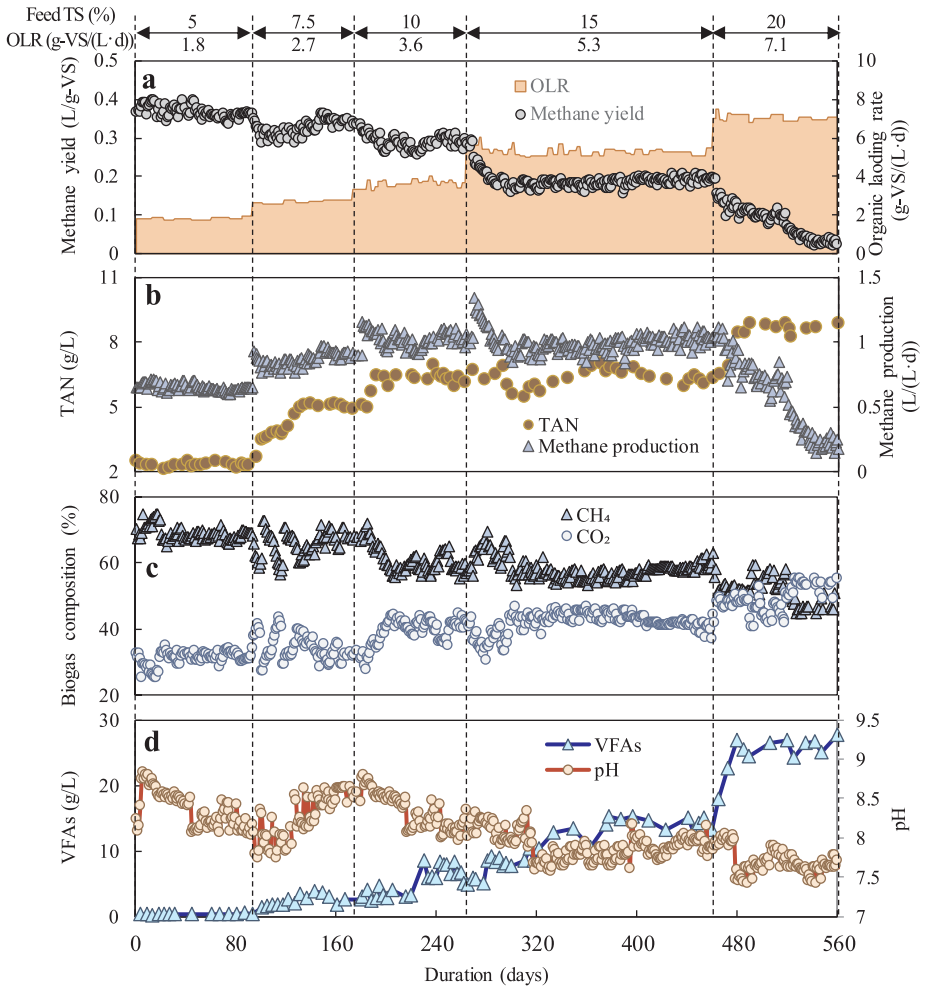

Fig. 2. The long term performance during 560 days of operation.

where R_{sample} is the tested ratio of heavy (^{13}C) to light (^{12}C) carbon; $R_{standard}$ is 0.0112372; δ_{CH_4} is the measured variable; δ_{ma} is the isotopic value of methane produced via acetate cleavage pathway and an average value of −33‰ was chosen; δ_{mc} is the isotopic value of methane produced via a carbon dioxide reduction pathway. The average values of δ_{ma} and δ_{mc} were chosen as previously described by Yin et al. (2018).

2.5. Microbial community analysis

Molecular analyses samples were collected from each stage on days 75–77, 160–162, 240–242, 330–332 and 556–558. These were then stored at −20 °C until use. The method of cetyltrimethylammonium bromide/sodium dodecyl sulfate was utilized for DNA extraction. The diluted DNA (10 ng/μL) was used (after purification by 1% agarose gel) in PCR for 16S rRNA genes amplification as described in a previous study (Algapani et al, 2018). PCR products were purified, quantitated, and then paired-end sequenced (2 × 300) by the platform of Illumina MiSeq. The raw sequence data was analyzed based on Bolger, et al., (2014), avoiding the poor-quality sequences. Subsequently, the raw data was analyzed using Majorbio I-Sanger Cloud software (www.i-sanger.com). The sequences were rarefied to 25,000 sequences per sample. And then grouped (at 97% similarity) into operational taxonomic units (OTUs). The DNA sequences were deposited at the database of Sequence Read Archive (SRA) with the number of PRJNA553511. The correlation between process parameters and microbial structure profile was determined using Redundancy Analysis (RDA) with software Canoco 5.0.

3. Results and discussion

3.1. Process performance under wet, high solid, and dry conditions

The volumetric methane production increased from 0.64 L/(L·d) in the wet system (TS 5%, OLR 1.8 g-VS/(L·d)) to 1.01 L/(L·d) in the high solid system (TS 15%, OLR 5.3 g-VS/(L·d)) but decreased to 0.14 L/(L·d) for the dry system (TS 20%, OLR 7.1 g-VS/(L·d), Fig. 2). Even though the volumetric of biogas was relatively stable during the operation at an OLR of 2.7–5.3 g-VS/(L·d), the specific methane yield gradually decreased over time (Fig. 2a). The methane content also decreased from 68% at 1.8 g-VS/(L·d) to 42% at 7.1 g-VS/(L·d) (Fig. 2c). The TAN concentration increased from 2.3 g/L to 8.5 g/L along with an increased OLR from 1.8 g to VS/(L·d) to 7.1 g-VS/(L·d) (Fig. 2b).

In the present study, the performance at each OLR stage during steady states was comparable with previous studies treating chicken manure (Table 1). The comparison results from the above mentioned studies clearly demonstrate the challenges with high VFA levels and reduced methane yield at an OLR over around 2.6 g-VS/(L·d). Ammonia inhibition is one of the most common reasons for process disturbance during degradation of protein-rich material. However, it is difficult to separate the effect of TAN inhibition from that of feeding TS and OLR as these factors are interrelated. VFA accumulation has been found to cause additional stress on the process and this has contributed to the risk of process failure at an elevated OLR (Bayrakdar et al., 2017). In

Table 1
Comparison of process performance obtained in this study with pervious works focusing on mesophilic AD of chicken manure in a CSTR.

Operating time (Days)	Feeding TS (%)	HRT (Days)	OLR (g-VS/(L·d))	Reactor working volume (L)	Temperature (°C)	TAN (g/L)	Free ammonia* (g/L)	VFA (g/L)	Methane yield (L/g-VS)	pH	References
161	2	29.2	0.45	5	35	1.1	0.03	NA	0.24	7.42	Webb and Hawkest, 1985
161	4	29.2	0.9	5	35	2.4	0.12	NA	0.26	7.68	Webb and Hawkest, 1985
161	6	29.2	1.4	5	35	3.3	0.24	NA	0.25	7.84	Webb and Hawkest, 1985
140	7	29.2	1.7	5	35	3.1	0.22	NA	0.21	7.84	Webb and Hawkest, 1985
140	10	29.2	2.5	5	35	4.5	0.43	NA	0.20	7.97	Webb and Hawkest, 1985
382	6	14.6	2.8	5	35	2.8	0.13	NA	0.24	7.65	Webb and Hawkest, 1985
165	10.4	25	3	20	37	3.0	0.16	6.0	0.24	7.7	Wu et al., 2016
40	5.7	30	1.3	12	35	4.0	0.26	1.0	0.30	7.80	Niu et al., 2013
125	10.5	30	2.6	12	35	5.0	0.69	1.5	0.21	8.15	Niu et al., 2013
59	11	30	2.7	12	35	8.0	1.33	5.0	0.18	8.25	Niu et al., 2013
19	16.9	30	3.8	5	36	6.9	0.68	7.5	0	7.96	Bayrakdar et al., 2017
233	NA	30	3.7	14	36	6.0	NA	34.3	0.05	NA	Molaey et al., 2018
93	5	20	1.8	12	37	2.3 ± 0.4	0.41 ± 0.07	0.4 ± 0.1	0.36 ± 0.01	8.21 ± 0.12	This study
87	7.5	20	2.7	12	37	5.0 ± 0.4	1.42 ± 0.11	2.2 ± 0.9	0.34 ± 0.02	8.49 ± 0.12	This study
90	10	20	3.6	12	37	6.5 ± 0.5	0.81 ± 0.06	6.7 ± 1.2	0.28 ± 0.02	8.04 ± 0.17	This study
193	15	20	5.3	12	37	6.9 ± 0.4	0.65 ± 0.04	13.6 ± 2.7	0.19 ± 0.02	7.91 ± 0.12	This study
97	20	20	7.1	12	37	8.5 ± 0.4	0.51 ± 0.02	25.2 ± 1.6	0.02 ± 0.01	7.70 ± 0.20	This study

*: free ammonia was calculated according to Bi et al., 2019b; TS: total solid; HRT: hydraulic retention time; TAN: total ammonium nitrogen; OLR: organic loading rate; VFA: volatile fatty acid; NA: data not available.

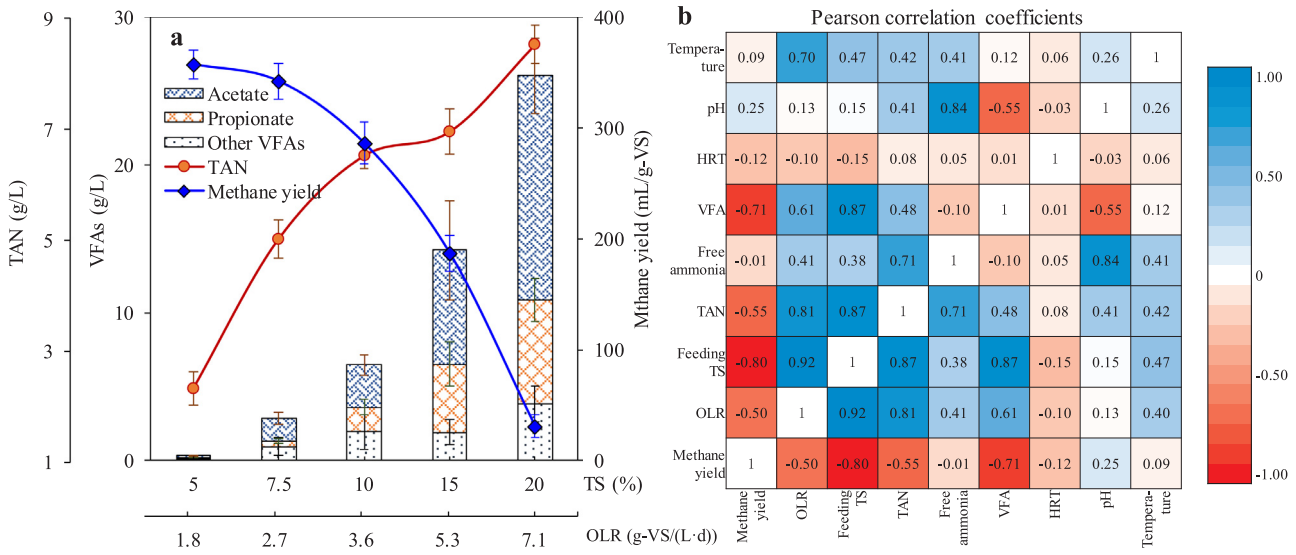

Fig. 3. Correlation between feeding total solid (TS), organic loading rate (OLR), total ammonia–nitrogen (TAN), volatile fatty acid (VFA) level, temperature, free ammonia, pH and methane yield.

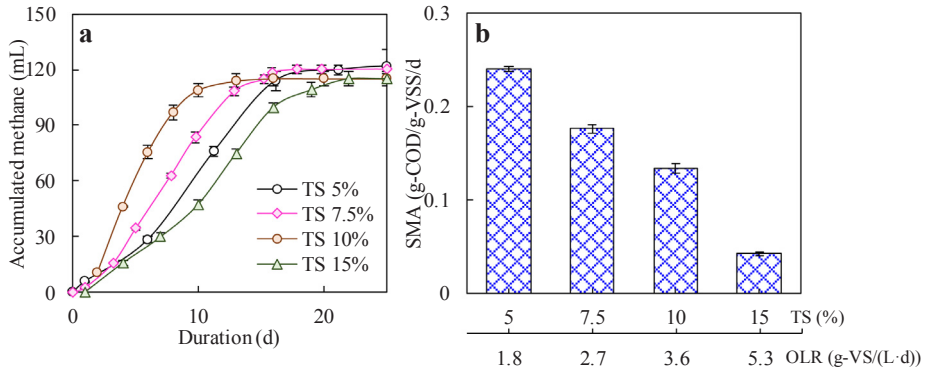

Fig. 4. Accumulated methane (a) and specific methanogenic activity (b) in sludge taken from digesters when operating at an OLR of 1.8–5.3 g VS/(Ld) (corresponding to 5–15% TS content in feedstock).

Table 1, digester performance revealed a comprehensive response to various operating parameters, i.e. TAN, VFA, feeding TS, and OLR. In the present study, calculation of the PCA results showed that feeding TS, OLR and VFA negatively correlated (*P* less than 0.05) with methane yield, whereas TAN and VFA positively related (*P* less than 0.05) with the feeding TS and OLR (Fig. 3b).

Comparisons from the aforementioned studies of the methane yield at mesophilic ranges can be found in Table 1. These results indicate that the AD of chicken manure gives an acceptable methane yield when operated at an OLR below 3 g-VS/(L·d) and a HRT of 30 days (Webb and Hawkest, 1985; Niu et al., 2013). Using a similar OLR, the current study demonstrated that a shorter HRT (20 days) can give a high methane yield of 0.34–0.36 L CH4/g VS (Fig. 3a). However, when the OLR increases above 3 g-VS/(L·d), a HRT of 20–30 days appears more troublesome, with raised VFA levels and reduced methane yield (present study, Bayrakdar et al., 2017; Molaey et al., 2018, Table 1). The comparisons therefore suggest that the nitrogen-rich characteristic makes the AD of chicken manure more difficult at a higher OLR.

3.2. Variation of specific methanogenic activities

The specific methanogenic activities (SMA) analysis showed a close relatedness between loss of methanogenic activity and reduced methane yield in the digester at a higher OLR (Fig. 4). When operating at an OLR of 2.7–5.3 g-VS/(L·d), results showed that 83%-86% of the total acetate added (4 g-COD/L) was converted to methane (Fig. 4a), indicating a theoretical conversion coefficient of 350 mL-CH_4/g-COD under standard temperature and pressure (273.15 K, 100 kPa). Even though all conditions had a similar total accumulated methane yield after about 20 days of incubation, the methane production rate differed considerably depending on the OLR and TAN-level. The SMA deceased from 0.24 to 0.18, 0.13, and 0.04 g-COD/g-VSS/d when the OLR increased from 1.8 to 2.7, 3.6, and 5.3 g-VS/(L·d) and the TAN increased from 2.3 to 5.0, 6.3, and 6.9 g/L (Fig. 4b, Table 1). A comparative SMA value, i.e. 0.25 g-COD/g-VSS/d was reported in the AD of chicken manure at an OLR of 1.6 g-VS/(L·d) and a TAN of 2.5 g/L (Bi et al., 2019a). Decreases in the SMA (39%-40%) have also been previously demonstrated in the mesophilic AD of chicken manure at TAN levels between 1.2 and 5.5 g/L when the OLR was increased (Bi et al., 2019a; Sung and Liu, 2003). Lower SMA values, i.e. 0.08 and 0.03 g-COD/g-VSS/d have been reported for the AD of chicken manure at a TAN of 4.0 and 6.0 g/L (Wandera et al., 2018). In this study, methane was not produced in sludge taken from the digester operated at an OLR of 7.1 g VS/L d, which correlates with the small amount of methane production in the digester at this stage. The decrease in the SMA was also related to the changes in methanogenic pathways and the microbial structure, as the SAO-HM pathway and *Methanosarcina* and *Methanoplasma* predominated during higher feeding TS and OLR (details are discussed in section 3.3). Additionally, the lower methanogenic activity, which occurred as a result of the higher ammonia and OLR levels, was likely a

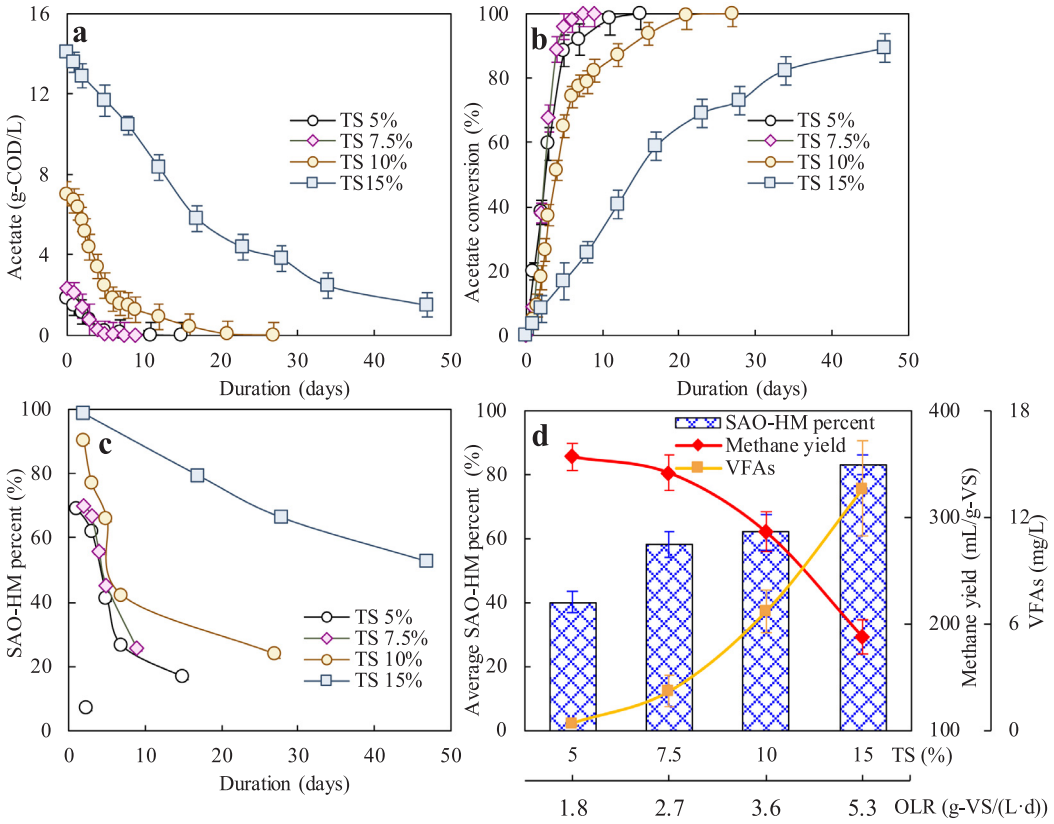

Fig. 5. Acetate degradation and contribution of the syntrophic acetate oxidation (SAO-HM) pathway for methanogenesis of acetate at a different organic loading rate (OLR) and total solid (TS) content in feedstock.

Table 2
Effect of total ammonium nitrogen (TAN) on methanogenic pathway in biogas digesters operating at mesophilic temperature conditions.

Feedstock	Temperature (°C)	OLR	TAN (g/L)	Acetate (g/L)	Percent of SAO-HM (%)	Reference
Chicken manure	37	1.7	2.5	2	41	Yin et al., 2018
Food waste	36	NA	0.2–1.6	NA	9–23	Jiang et al. 2017
Food waste	36	NA	4.6–6.1	NA	68–75	Jiang et al. 2017
Distiller's grains	38	11.0	3.5–3.7	NA	46–54	Mulat et al., 2016
Chicken manure	37	1.8	2.5	2	40 ± 3	This study
Chicken manure	37	2.7	5	2.4	58 ± 3	This study
Chicken manure	37	3.6	6.5	7	62 ± 3	This study
Chicken manure	37	5.3	6.9	14	83 ± 3	This study

SAO-HM: syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis; NA: data not available.

Table 3
The bacterial community (Phyla and Class level) under different operating conditions.

Phyla	Class	Bacterial related abundance (%)				
		Wet AD system			High solid AD system	Dry AD system
		TS_{in} 5% OLR 1.8	TS_{in} 7.5% OLR 2.7	TS_{in} 10% OLR 3.6	TS_{in} 15% OLR 5.3	TS_{in} 20% OLR 7.1
Firmicutes	Clostridia	41.1	44.4	36.2	33.5	42.4
Firmicutes	Bacilli	0.1	1.3	4.9	2.9	2.4
Firmicutes	Erysipelotrichi	0.1	0.3	6.8	7.1	0.7
Bacteroidetes	Bacteroidia	19.9	24.1	32.8	20.1	14.9
Bacteroidetes	Flavobacteriia	0.1	0.1	0.1	0.1	5.3
Proteobacteria	Gammaproteobacteria	16.3	14.7	1.8	9.5	7.1
Cloacimonetes	Cloacamonae	0.4	0.6	9.1	14.9	10.5
Tenericutes	Mollicutes	0.8	0.7	3.2	3.4	3.7
Spirochaetes	Spirochaetes	2.4	0.9	1.1	2.4	3
Actinobacteria	Actinobacteria	0.1	0.1	2.6	2.3	3.1
Others*	Others*	18.9	12.9	1.4	3.8	6.9

*: others is the sum of the microorganisms less than 1%. The unit of OLR was g- VS/(L·d).

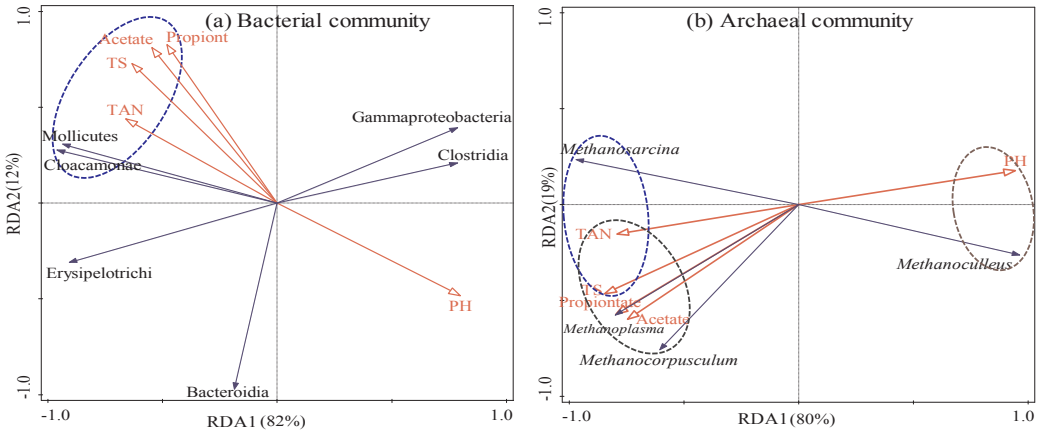

Fig. 6. Redundancy analysis of the operational parameters (total ammonia nitrogen (TAN), total solid content in feedstock (TS), acetate, propionate, and pH) and (a) bacterial and (b) methanogenic microbial communities.

Table 4
Changes of syntrophic acetate-oxidizing bacteria (SAOB) and methanogenic community (genus level) under wet, high solid, and dry AD.

	Genus	Related abundance (%)				
		Wet AD system			High AD system	Dry AD system
		TS_{in} 5% OLR 1.8	TS_{in} 7.5% OLR 2.7	TS_{in} 10% OLR 3.6	TS_{in} 15% OLR 5.3	TS_{in} 20% OLR 7.1
SAOB	*[Clostridum]*	/	0.8	0.7	0.1	0.1
	Tepidanaerobacter	/	0.3	/	/	/
	Syntrophaceticus	0.1	0.1	/	0.1	/
Mixture-trophic methanogens	*Methanosarcina*	/	/	93.7	72.5	36.5
Hydrogenotrophic methanogens	*Methanoculleus*	98.1	98.8	1.9	3.9	5.1
	Methanocorpusculum	1.5	0.1	/	11.1	12.3
	Methanoplasma	/	/	0.9	8.7	42.2
	Methanobrevibacter	/	/	1.4	2.7	3.2
Others*	Others*	0.4	1.1	2.2	1	0.7

SAOB: syntrophic acetate-oxidizing bacteria; Mixture-trophic: methanogens can utilize acetate and hydrogen; '*': others is the sum of the methanogens less than 1%; '/'is the microorganisms below the detected level. The unit of OLR was g- VS/(L·d).

direct cause of the gradual rise in acetate levels as the digester OLR increased. The elevated acetate in AD process as a 2.0 g/L acetate has been reported to have a negative effect on the degradation of propionate (Mawson et al., 1991). Therefore, the accumulation of VFA may be a result of low methanogenic activity when the digester is operated at a high OLR and TAN.

3.3. Shifts of methanogenic pathways and microbial community

The pathway for the methanisation of acetate was quantitatively analyzed by evaluating the value of f_{mc}, i.e., the proportion of CH_4 obtained by CO_2 reduction. In Fig. 5a it can be seen that most of the acetate (93%-100%) was degraded within 48 days of incubation. The slower methane production rate was observed in the high solid AD digester, as is shown in Fig. 5b. Both ammonia and acetate have been identified as selective factors in the shift of the methanogenic pathway from acetoclastic methanogenesis to SAO-HM (Westerholm et al., 2016; Hao et al., 2011). The decline of the SAO-HM percentage with the degradation of acetate is represented in Fig. 5c. A sharper decrease in the percentage was observed in the digesters fed with diluted chicken manure. Under those conditions, the ammonium concentration was kept at relative levels.

In the present study, the average percent of methane production through SAO-HM rose from 40% to 80% with the increased OLR (from 1.8 to 5.3 g-VS/(L·d)) (Fig. 5d). As summarized in Table 2, the percentage of SAO-HM methanogenesis rose as both ammonia and acetate increased in the AD of food waste, chicken manure and distiller's grains (Table 2). Consequently, the elevated ammonia concentration, and the shift of methanogenic pathways with the increase of OLR, are suggested as causes of the reduction in methanogenic activity and methane yield.

The methanogenic pathway shifts may fundamentally originate from the changes in the microbial communities. The Phyla and Class bacterial structure at wet, high solid, and dry AD systems are provided in Table 3. The *Clostridia* (phylum *Firmicutes*), *Bacteroidia* (phylum *Bacteroidetes*), and *Gammaproteobacteria* (phylum *Proteobacteria*) were the most dominant bacterial classes in those systems. A significant rise in *Cloacamonae* (phylum *Cloacimonetes*) from 0.4% to 15% was found with the increase of the total solids in the feeding of the digesters. Redundancy analysis (RDA) showed positive correlations between the relative abundance of *Cloacamonae* and the OLR, TAN and VFA concentration (Fig. 6a). The *Cloacimonetes* was reported to decompose protein, cellulose, and lipids and its ability to perform propionate-oxidization was also suggested (Dyksma et al., 2019; Guo et al., 2015). The dominance of *Cloacimonetes* was observed when an AD process was disturbed by high ammonia in the AD of fish waste (Solli et al., 2014) and chicken manure (Klang et al., 2019). The genera ([*Clostridium*], *Syntrophaceticus* and *Tepidanaerobacter*), which may include the known mesophilic syntrophic acetate-oxidizing bacteria (Westerholm et al., 2010; Westerholm et al., 2019), were only found at a low relative abundance, as illustrated in Table 4. However, those low abundant bacterial play an important role in the AD system when considering the dominance of the SAO-HM methanogenesis.

The significant changes in the methanogens structure can be observed in Table 4. The genus *Methanoculleus,* which is involved in the

SAO-HM pathway (Westerholm et al., 2019), clearly dominated in the wet AD digesters fed with 5% and 7.5% TS chicken manure (Table 4). However, a sharp decrease of the *Methanoculleus* was found when the total solid in feeding increased to 10%, the highest TS in the wet AD in this study. The *Methanoculleus bourgensis* was reported as a high tolerance methanogen (Maus et al., 2015), and a low growth rate of *Methanoculleus* (doubling time, 23–50 d) was observed under a TAN level of 4.8 g/L TAN (Westerholm et al., 2019). In this study, *Methanoculleus* did not appear to sustain its metabolic ability at a HRT of 20d when the OLR increased. As can be seen in Table 4, the dominance of *Methanoculleus* was replaced by the genus *Methanosarcina* when the digester was fed with 10%, 15% and 20% TS chicken manure. The dominance of *Methanosarcina* under high ammonium and OLR conditions may indicate may indicate that it has a high tolerance under such stressed conditions. *Methanosarcina* has the ability to produce methane through both the AM (as acetoclastic methanogen) and SAO-HM pathway (as hydrogen scavenger) (Vrieze et al., 2012), and *Methanoplasma* and *Methanocorpusculum* increased to 42.2% and 12.3% in the dry AD digester. *Methanosarcina* was found to be a hydrogen scavenger in SAO-HM, and *Methanoplasma* and *Methanocorpusculum* were hydrogenthopic methanogens (Lang et al., 2015). Positive correlations between the relative abundance of *Methanocorpusculum* and *Methanoplasma,* and concentrations of TAN and VFA, were also observed in the current study (Fig. 6b). In contrast, a negative correlation between the relative abundance of *Methanoculleus* and VFA was observed. Overall, therefore, the shift in the dominant methanogens reflected the pressure of the ammonium and OLR on the process performance. Additionally, the SAO-HM pathway occurred when the system encountered stressed conditions and responded to the shift in the microbial community.

4. Conclusions

The AD of chicken manure was challenged under high solid conditions and the performance deteriorated under a dry system even through long term acclimation. The quantitative methanogenic pathway and microbial community results indicated that the SAO-HM plays a more important role in both high solid and dry AD systems but it cannot sustain a satisfactory performance. At a fixed 20-day HRT, both the step-increased ammonia levels and the high OLR seemed to be most significant in inducing process disturbance. If lowering the OLR can alleviate the inhibition under the ammonia-stressed conditions need to be further investigated.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.122342.

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