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Impact of the substrate loading regime and phosphoric acid supplementation on performance of biogas reactors and microbial community dynamics during anaerobic digestion of chicken wastes

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HIGHLIGHTS

- Anaerobic digestion of chicken wastes under various conditions was investigated.
- Phosphoric acid at moderate level positively affected the anaerobic digestion process.
- 454 pyrosequencing approach was used to evaluate the microbial community diversity.
- *Bacteroidales*, *Erysipelotrichaceae*, *Clostridium*, *Methanosarcina* were the abundant taxa.
- The major process parameter shaping community structure was the high ammonia level.

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ABSTRACT

This study evaluates the effects of increasing organic loading rate (OLR) and decreasing hydraulic retention time (HRT) as well as phosphoric acid addition on mesophilic reactors' performance and biogas production from chicken wastes. Furthermore, microbial community composition in reactors was characterized by a 16S rRNA gene-based pyrosequencing analysis. Each step of increasing OLR impacted on the activity of microorganisms what caused a temporary decrease in biogas production. The addition of phosphoric acid resulted in the increased biogas production with values between 361 and 447 mL g_{VS}^{-1} from day 61 to day 74 compared to control reactor (309–350 mL g_{VS}^{-1}). With reactors' operation, *Bacteroidetes* phylotypes were noticeably replaced with *Firmicutes* representatives, and significant increase of *Clostridium* sp. was identified. Within *Euryarchaeota*, *Methanosarcina* sp. dominated in all analyzed samples, in which high ammonium levels were detected (3.4–4.9 NH_4^+-N g L^{-1}). These results can help in better understanding the anaerobic digestion process of simultaneously ammonium/phosphate-rich substrates.

1. Introduction

Anaerobic digestion has received significant importance within the last 20–30 years. Biogas as a representative of renewable energy sources can be obtained via biological degradation of various organic waste materials (e.g. agricultural residues and municipal solid wastes) under anaerobic conditions. This anaerobic process leads to organic waste utilization simultaneously with the energy generation, thus solving society's most urgent needs, effective wastes disposal and alternative clean energy production. Research activities in the field of anaerobic digestion processes have recently received much attention, particularly focusing on the influence of different operational and environmental parameters on different types of biogas reactors' performance, optimization of anaerobic digestion processes and microbiology of anaerobic digesters (Niu et al., 2013a).

Anaerobic treatment of poultry manure is more problematic than anaerobic digestion of manure from other farm animals, since poultry wastes contain high amounts of nitrogen and their anaerobic conversion can result in ammonia inhibition process. Anaerobic digestion of chicken wastes with high content of uric acid and undigested proteins results in the production of toxic un-ionized free ammonia (NH_3, FAN) and ionized ammonium ions

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(NH_4^+). Nitrogen is a macronutrient that is required in large quantities by bacteria and methanogenic archaea. However, even some ammonium compounds are beneficial for microbial growth, free ammonia above critical concentrations has been considered as the cause of inhibition of microorganisms during anaerobic digestion of nitrogen-rich substrates (Abouelenien et al., 2010). Especially ammonia is the most significant inhibitor of the last stage, methanogenesis, and therefore high amounts of NH_3 can decrease overall specific methane production (Niu et al., 2013b; Lv et al., 2014). Different environmental parameters, mostly rising temperature and pH, impact on balance of NH_3/NH_4^+ (total ammonia nitrogen, TAN) and can lead to the increased effect of toxicity of ammonia on anaerobic microorganisms (Garcia and Angenent, 2009). In addition to toxicity, chicken wastes contain a fraction of wood chips and shavings used as the bedding materials, and therefore such wastes with high NH_3 and lignocellulosic content are less amenable to anaerobic destruction with biogas production (Costa et al., 2012; Ziganshina et al., 2014a).

Anaerobic digestion depends on the coordinated activity of various microbial groups and involves several consecutive destruction stages, such as hydrolysis, acidogenesis, acetogenesis and finally methanogenesis. The activity and fate of distinct groups of microorganisms make the study of anaerobic digestion more challenging. The last step of anaerobic digestion consists of aceticlastic, hydrogenotrophic and methylotrophic methanogenesis (Demirel and Scherer, 2008). Since aceticlastic methanogenic archaea are more susceptible to process disturbances during anaerobic digestion, hydrogenotrophic methanogenesis is considered to be the main methanogenic pathway at high ammonia concentrations. The released acetate is biodegraded via syntrophic acetate oxidation (SAO) pathway, and SAO coupled with hydrogenotrophic methanogenesis has been reported to be the predominant pathway at high ammonia levels in distinct anaerobic reactor systems (Schnürer and Nordberg, 2008; Fotidis et al., 2014). On the contrary, aceticlastic methanogenesis performed by the activity of acclimatized *Methanosarcinaceae* at high ammonium (5–7 NH_4^+-N g L^{-1}) and acetate (9 g L^{-1}) concentrations was recently demonstrated in batch tests (Fotidis et al., 2013). Therefore, the influence of different ammonia concentrations on the methanogenic pathways and microbial community structure should be further evaluated. In addition, it is important to enrich or construct highly adapted bacterial and archaeal communities which can be used as remedy in case of inhibition of anaerobic digestion process.

To avoid inhibition effects during anaerobic digestion of ammonium-rich organic wastes, some research works have explored different techniques for digesting such problematic wastes. Co-digestion of chicken manure with other substrates can be one strategy to control ammonia inhibition (Wang et al., 2012). In another work, the addition of zeolites to an anaerobic reactor enhanced methane gas yield and efficiently removed TAN released during anaerobic digestion of ammonium-rich swine wastes (Wang et al., 2011). Abouelenien et al. (2010) showed that ammonia released during anaerobic conversion of chicken manure in thermophilic batch-mode treatments was effectively removed during recycling of biogas followed by gas washing in sulfuric acid to capture ammonia. Nie et al. (2015) reported that technical stripping effectively removed free ammonia from the liquid fraction of the effluent of mesophilic anaerobic reactor at high organic loading rate (OLR of 5.3–6.0 g_{VS} L^{-1} d^{-1}). In another work, the reduction of the piggery wastewater pH from its initial pH of 8.3 to 6.5 resulted in the stimulation of methane production coupled with the decrease in FAN concentrations in batch thermophilic experiments (Ho and Ho, 2012). Therefore, the reduction of ammonia levels in anaerobic digesters operating at high TAN levels is preferable to decrease the free ammonia inhibition.

Except nitrogen, phosphorous is a macronutrient that is required in relatively large quantities by bacteria and methanogenic archaea, playing an important role in their metabolism. It was previously established in batch tests that the addition of phosphate at values within 414–465 mg P L^{-1} accelerated the anaerobic digestion processes (Lei et al., 2010; Wang et al., 2015). However, these experiments were performed in the presence of low ammonia concentrations, indicating the necessity to conduct additional experiments devoted to anaerobic conversion of simultaneously phosphorus-rich and ammonium-rich substrates. In addition, it is significant to evaluate the effect of phosphate/phosphoric acid addition on the activity of microbial communities during anaerobic digestion of such wastes. Furthermore, co-digestion of various ammonium-rich and phosphorus-rich substrates can be used to improve the quality of fertilizers. Since free ammonia levels drop in parallel with pH fall, the addition of phosphoric acid to digesting mixture can be considered as another strategy to stabilize pH and also to reduce FAN concentrations in the ammonium-rich anaerobic digestion systems.

In the present study, the first goal was to study the effect of increasing OLR and decreasing HRT on mesophilic continuously stirred tank laboratory reactors' performance during anaerobic digestion of ammonium-rich chicken wastes. The second goal was to gain insight regarding the influence of phosphoric acid addition on the efficiency of biogas production from chicken wastes. Finally, bacterial and archaeal community structure and dynamics in lab-scale reactors were characterized using a 16S rRNA gene amplicon 454 pyrosequencing approach. The main factors affecting microbial community shifts were also evaluated by nonmetric multidimensional scaling analysis.

2. Methods

2.1. Chicken wastes properties

The chicken wastes with total solids (TS) of 78.28 ± 0.4%, volatile solids (VS) of 69.79 ± 0.7%, total nitrogen of 3.35 ± 0.11% and total phosphorous of 3.04 ± 0.12% were collected from a poultry farm located in the Zelenodolsky district, the Republic of Tatarstan (Russia) and stored in a refrigerator at 4 °C. These wastes contained chicken manure and a fraction of chicken feathers with wood shavings. After calculating the necessary OLR and HRT, the desired amount of chicken wastes were diluted with tap water, thoroughly stirred and transferred into continuously stirred tank laboratory reactors (CSTR).

2.2. CSTRs operation procedure

Two continuously stirred tank laboratory reactors with an active volume of 10 L (total 12 L) were operated under mesophilic conditions (38 °C). The height of the reactors was 33 cm and the diameter was 21 cm. Both reactors were warmed by water circulation and agitated continuously with a motor at 60 rpm. Initially, fresh cow manure (4.6 kg) was mixed with tap water (5.4 L) and then used for the experiments start-up. After less than 1 month, the biogas production from cow manure was reduced, and then reactors were daily supplied only with chicken wastes. Chicken wastes were diluted with tap water and further used as the substrate for the whole experiment (137 days). The first reactor served as a control reactor (R_C), while the second reactor (R_PA) additionally operated with phosphoric acid (85% H_3PO_4) during a period of 32 days (days 61–92 days). Reactor R_PA was daily supplied with 1.4 mL of 85% H_3PO_4 from day 61 to day 74 and with 2.0 mL of 85% H_3PO_4 from day 75 to day 92. Phosphoric acid was added to R_PA jointly with the chicken wastes. The OLR in R_C

was gradually increased from 1.0 to 3.2 $g_{VS}\,L^{-1}\,d^{-1}$ during the operating period. The OLR in R_PA was firstly increased from 1.0 to 2.8 $g_{VS}\,L^{-1}\,d^{-1}$ (days 1–99), but then chicken wastes were not added to R_PA during a 3-day period (days 100–102) because of the accumulation of high amounts of organic acids. After this period, the OLR in reactor R_PA was raised to 1.5 $g_{VS}\,L^{-1}\,d^{-1}$ and kept constant until day 110. The next increase of the OLR to 2.3 $g_{VS}\,L^{-1}\,d^{-1}$ (days 111–115) successively stabilized the process. Finally, the OLR in R_PA was increased to 3.2 $g_{VS}\,L^{-1}\,d^{-1}$ and it functioned as well as R_C. The effluent was withdrawn simultaneously with feeding of both reactors. Reactors operated at HRT of 50 days during a period of 89 days, whereas the HRT was then reduced to 40 days for the remaining operating period. Samples for microbial community analyses were collected at three distinct times: day 77, day 99 and day 127.

2.3. Analytical methods

Ritter milligascounters (MGC-1, Ritter, Germany) were used to analyze the amount of daily biogas production; values were corrected to standard pressure and temperature conditions. The specific biogas productions (SBP) and specific methane production (SMP) were presented as gas production per gram of influent VS. Biogas composition, including CH_4, CO_2 and H_2S concentrations, was analyzed using a GA 2000 gas analyzer (Keison, UK). Effluents were collected from both biogas reactors one or 2 times per week for analyses, including TS, VS, pH, volatile fatty acids (VFA), TAN and FAN. TS were determined by oven drying at 105 °C for 16 h, and VS were then analyzed by combusting the dry matter at 550 °C for 3 h. Acid capacity of the effluents was analyzed using a T90 Mettler-Toledo titrator (Switzerland) according to Ziganshin et al. (2012). In addition, soluble phosphate as well as propionate and acetate concentrations were determined with a Dionex ion chromatograph equipped with IonPac AS9-HC or IonPac AS11 columns (Ziganshin et al., 2010). TAN was analyzed in the liquid phase of the effluents by distillation and absorption in H_3BO_3 followed by titration with H_2SO_4. FAN concentration was calculated from the TAN concentration, pH and temperature values. Each chemical analysis represents the average value and standard deviation for at least three determinations.

2.4. DNA extraction and pyrosequencing

After centrifugation of the effluents (15 mL) at 20,000×*g* for 10 min, total metagenomic DNA was extracted from each sample using a FastDNA spin kit for soil (MP Biomedicals, USA). The quantity of the extracted DNA was measured by using the Qubit dsDNA BR assay kit and a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). The ~500-bp fragments of the bacterial 16S rRNA gene were amplified from each DNA sample using universal primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGG CAC-3′), whereas amplification of ~450-bp fragments of the archaeal 16S rRNA gene was accomplished using universal primers Arch349F (5′-GYGCASCAGKCGMGAAW-3′) and Arch806R (5′-GGA CTACVSGGGTATCTAAT-3′). These primer pairs were tailored by adding a fusion linker and a 10-nucleotide barcode sequence at the 5′ end of the forward primer and a fusion linker sequence at the 5′ end of the reverse primer. The reactions were run in triplicate for each sample using the FastStart High Fidelity PCR System (Roche, Branford, CT). The received amplicons from each sample were combined and purified with the Agencourt AMPure XP system (Beckman Coulter, Brea, CA); the purified amplicons were further quality-checked using the high sensitivity DNA kit and an Agilent 2100 Bioanalyzer system (Santa Clara, CA). Fluorometric quantitation and preparation of the amplicon library were performed according to the GS Junior Amplicon Library Preparation Method Manual (Roche). Bacterial and archaeal amplicons were pooled separately and then subjected to emulsion PCR by using the Lib-L emPCR Kit (Roche). The 16S pyrosequencing runs were performed on a GS Junior picotiter plate according to the manufacturer's recommendations.

2.5. Analysis of sequences and statistical analysis

Analysis of raw data with further sorting of the multiplex identifiers were performed using the GS Junior software. The 16S pyrosequencing reads were further processed and analyzed using the bioinformatics package QIIME, version 1.8.0 (http://qiime.org/) in accordance with QIIME protocols. Aligned sequences were clustered at 97% sequence similarity. Observed operational taxonomic unit (OTU) numbers, Shannon, Simpson and Chao 1 diversity indices, as well as the phylogenetic distance were estimated as the indicators for alpha diversity. The beta diversity of microbial community within the reactors' samples was compared by using principal coordinate analysis (PCoA) on unweighted UniFrac distances implemented in QIIME. In addition, a multivariate statistical analysis of the normalized diversity tables was run with the R package vegan. Nonmetric multidimensional scaling (NMDS) analysis with the appliance of the Bray–Curtis similarity index was used to plot the rank order of similarity of assigned bacterial and archaeal reads. The major anaerobic process parameters and their correlations with the microbial community diversity were fitted with the envfit function in the Vegan package. The significance of single experimental parameters on the NMDS results was tested using a Monte Carlo permutation test with 999 permutations.

2.6. Nucleotide sequence accession numbers

The filtered pyrosequencing reads were deposited in the MG-RAST server (http://metagenomics.anl.gov/) under accession numbers 4602384.3 (R_C; day 77), 4602391.3 (R_C; day 99), 4602400.3 (R_C; day 127), 4602393.3 (R_PA; day 77), 4602395.3 (R_PA; day 99), 4602403.3 (R_PA; day 127) for bacteria sequences and 4602405.3 (R_C; day 77), 4625156.3 (R_C; day 99), 4602387.3 (R_C; day 127), 4602385.3 (R_PA; day 77), 4625157.3 (R_PA; day 99), 4602389.3 (R_PA; day 127) for archaea sequences.

3. Results and discussion

3.1. Process stability and biogas production

The efficiency of both control and experimental reactors' (R_C and R_PA) performance was evaluated by wastes destruction with the concomitant gas/methane production and accumulation of organic acids and TAN/FAN. The experiment was continued over 137 days to study the effect of increasing OLR and decreasing HRT on mesophilic reactors' performance during anaerobic digestion of ammonium-rich chicken wastes. Furthermore, the influence of phosphoric acid addition on performance of experimental reactor was investigated. It is also significant to investigate whether and how microorganisms are affected by high phosphate/phosphoric acid concentrations during anaerobic digestion of ammonium-rich substrate. The SBP, biogas composition, pH values, organic acids, TAN and FAN concentrations following operation time are shown in Figs. 1 and 2.

The whole experiment can be divided into 3 main stages. In stage I (60 days), chicken wastes as monosubstrate were added to both reactors to study reactors' performance during increasing OLR from 1.0 to 2.3 $g_{VS}\,L^{-1}\,d^{-1}$ and at constant 50-day HRT period. In this stage, the TAN accumulation up to 2.75 $g\,L^{-1}$ was observed. In stage II (32 days), the OLR was further increased from 2.3 to

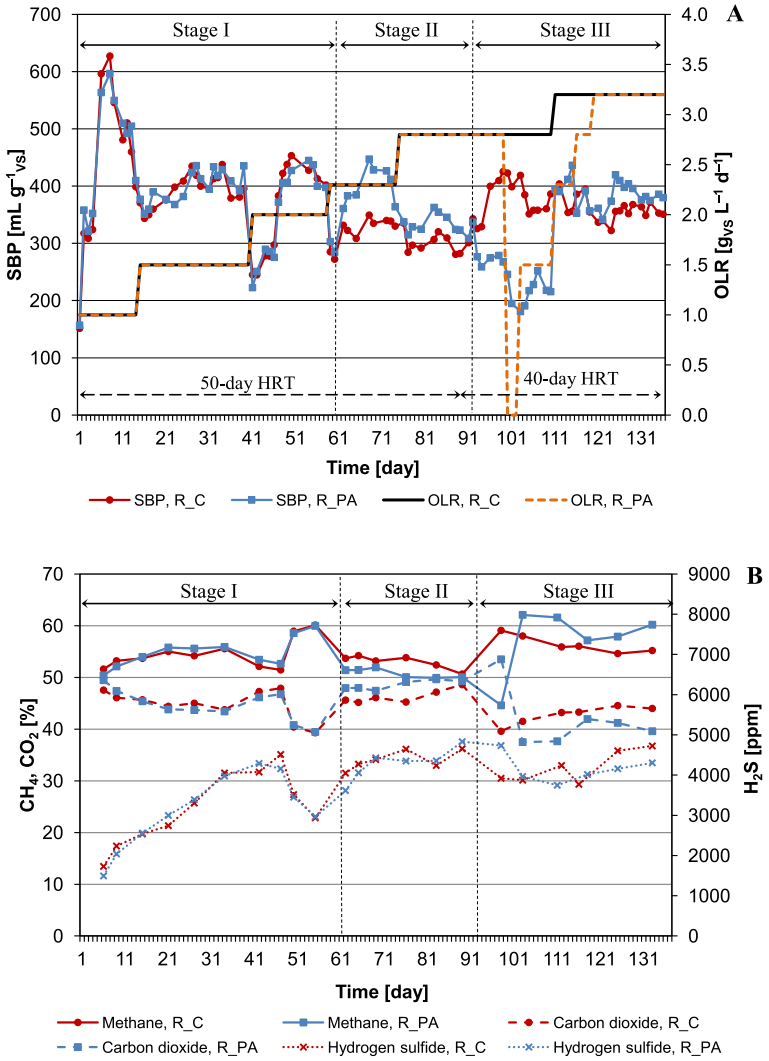

Fig. 1. (A) Specific biogas production (SBP) in reactors correlated to the organic loading rate (OLR) and hydraulic retention time (HRT). (B) Differences in methane, carbon dioxide and hydrogen sulfide concentrations in reactors. Reactors R_C and R_PA were fed with chicken wastes. Reactor R_PA was additionally supplied with H_3PO_4 during a 32-day experimental period (days 61–92; Stage II).

2.8 g_{VS} L^{-1} d^{-1}, and the 50-day HRT after 29 days was shortened to 40 days in both reactors. The simultaneous TAN accumulation up to 4.17–4.21 g L^{-1} during this stage was detected. In addition, during the second stage, reactor R_PA was additionally supplied with 85% phosphoric acid, while reactor R_C functioned without any additional additives. In stage III (45 days), the OLR achieved 3.2 g_{VS} L^{-1} d^{-1} value in both reactors at the end of the operating period. Furthermore, reactor R_PA functioned in the absence of phosphoric acid throughout this period to determine retention or loss the positive effect of the process performance after the period of inorganic acid addition.

In stage I, the SBP rate, biogas composition, pH values, VFA and TAN/FAN concentrations in both reactors were similar, indicating that reactors operated almost identically (Figs. 1 and 2). A sharp peak of SBP from both reactors during the 6–8 day experimental period at the OLR of 1.0 g_{VS} L^{-1} d^{-1} was observed, what could be related to various factors, including a better destruction of biomass at the beginning of the treatments. Increase of the OLR from 1.0 to 1.5 g_{VS} L^{-1} d^{-1} stabilized the biogas production rate, and reactors had a comparable SBP with maximum values of 435 and 439 mL g_{VS}^{-1} during days 27–40 for R_PA and R_C, respectively (Fig. 1A). In most cases, CH_4 accounted for 52–56% of biogas composition, CO_2 for 43–47% and H_2S for less than 4500 ppm (days 27–40) (Fig. 1B). During the 40-day experimental period, the pH, VFA and TAN levels in general increased (Fig. 2). Niu et al. (2013a) observed the similar biogas production rate in the presence of similar amounts of ammonium during mesophilic anaerobic treatment of stripped chicken manure. Further experiments showed that increasing OLR to 2.0 g_{VS} L^{-1} d^{-1} led to the SBP decrease in reactors (Fig. 1A), what was probably associated with the response to the increased OLR. In another work (Li et al., 2014), biogas and methane yields also relatively declined after the OLR was increased from 1 to 2 g_{VS} L^{-1} d^{-1} during anaerobic co-digestion of chicken manure and corn stover. The authors suggested that increasing OLR can impact on the activity of methanogenic archaea. In parallel, a sharp accumulation of organic acids in present study was detected (5.66 and 4.01 g L^{-1} in reactors R_C and R_PA at day 44, respectively) (Fig. 2A). The increase in VFA concentrations was consistent with an overall biogas and specific methane production rate decrease (Niu et al., 2013b; Lv et al., 2014). Further treatments resulted in more efficient VFA destruction with their better involvement in methanogenesis (days 47–58). Thus, the SBP achieved the similar values in both reactors (between 398 and 402 mL g_{VS}^{-1}) at day 58; the methane content increased up to 60% at day 56 (Fig. 1). When the OLR was further increased to 2.3 g_{VS} L^{-1} d^{-1}, the SBP in both systems again had dropped rapidly

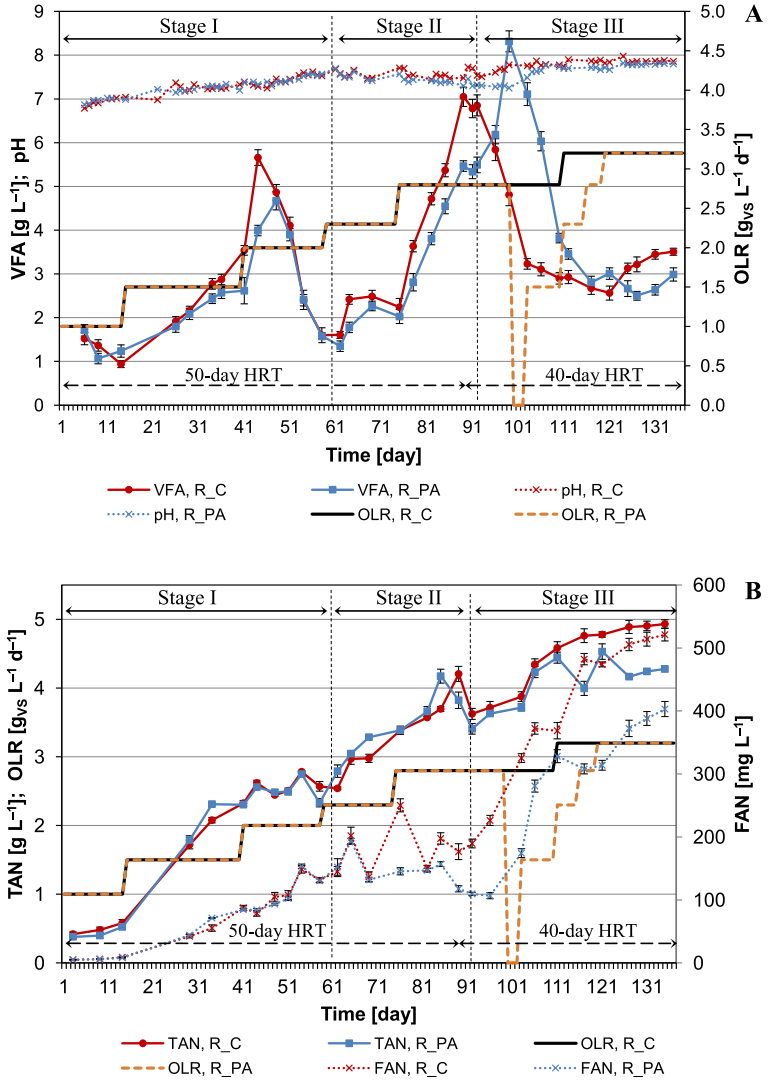

Fig. 2. (A) Volatile fatty acids (VFA) concentrations and pH in reactors correlated to the OLR and HRT. (B) Total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) concentrations in reactors correlated to the OLR and HRT. Reactors R_C and R_PA were fed with chicken wastes. Reactor R_PA was additionally supplied with H_3PO_4 during a 32-day experimental period (days 61–92; Stage II). *Error bars* represent the standard deviation of triplicate measurements.

(days 59–60; Fig. 1A), indicating that microorganisms were not adapted to higher OLR conditions (Li et al., 2014). The pH values of both reactors increased from ~6.8 to ~7.7 during the 60-day experimental period (Fig. 2A), suggesting that chicken wastes and the released ammonia contributed to the pH in reactors. In stage I, the TAN concentrations in reactors R_C and R_PA increased from initial 0.41 and 0.39 g L^{-1} to 2.57 and 2.33 g L^{-1} at day 58, accordingly (Fig. 2B).

In stage II, experimental reactor (R_PA) was additionally supplied with 1.4 mL of 85% H_3PO_4 from day 61 to day 74. As can be seen in Fig. 1A, the addition of phosphoric acid resulted in the increased SBP with values between 412 and 447 mL g_{VS}^{-1} from day 68 to day 73 compared to control reactor (335–350 mL g_{VS}^{-1}). In addition, the pH dropped from ~7.7 to 7.45, and organic acids were detected at lower values in R_PA during this stage (Fig. 2A). From day 75 to day 92, reactor R_PA was supplied with 2.0 mL of 85% H_3PO_4 because of the increasing OLR. When the OLR was further increased to 2.8 g_{VS} L^{-1} d^{-1}, the SBP in control and experimental reactors had dropped to 281–322 and 323–363 mL g_{VS}^{-1}, respectively (Fig. 1A). The volumetric biogas production (VBP) from R_PA achieved 284 L, whereas VBP from R_C was lower and achieved only 252 L during the 32-day experimental period (data not shown), indicating the effectiveness of the addition of phosphoric acid during anaerobic digestion of chicken wastes. Even a slightly higher methane level in biogas from reactor R_C was observed (Fig. 1), the SMP from R_PA was higher than that of R_C (data not shown). The pH in R_C was maintained between 7.6 and 7.7 values, while the pH in R_PA was changed to 7.2–7.3 (Fig. 2A). The VFA, TAN and FAN concentrations in reactor R_C increased to maximum 7.05, 4.21 and 0.18 g L^{-1} at day 89, respectively. The VFA, TAN and FAN levels in reactor R_PA also increased but to lower values of 5.46, 3.82 and 0.12 g L^{-1} at day 89, respectively (Fig. 2).

Organic acids accumulation will follow with ammonia inhibition, since during ammonia inhibition process the activity of aceticlastic methanogens are inhibited, leading to generally increasing levels of VFA (Niu et al., 2013b; Nie et al., 2015). In the inhibition stage, hydrolysis and acidogenetic activity in parallel with methanogenesis are also suppressed (Nielsen and Angelidaki, 2008). The interaction between NH_3, VFA and pH may lead to a process running stably but with a lower methane yield. However, continuous acidification finally can lead to anaerobic digestion process failure (Lv et al., 2014). After day 89, the HRT was shortened from 50 to 40 days, and the ammonium nitrogen values in R_C

and R_PA were reduced to 3.62 and 3.41 g L^{-1}, respectively. This decrease could have been influenced by the increasing dilution of substrate with water and by other factors what was also suggested by Schmidt et al. (2014). Finally, compared to R_C, R_PA performed more effectively during the stage of H_3PO_4 addition, what was reflected in a higher biogas yield, in more efficient VFA destruction with their better involvement in methanogenesis and in lower amounts of FAN detected.

Wang et al. (2015) in batch tests digesting sludge showed that the presence of phosphate salts at intermediate concentration (414 mg P L^{-1}) could accelerate aceticlastic methanogenesis, acetogenesis and acidogenesis, but the increase of the phosphate concentration demonstrated these processes down. Lei et al. (2010) also found that an adequate level of phosphate addition (465 mg P L^{-1}) could accelerate the biogasification process from rice straw and sludge in batch experiments. However, all these experiments were conducted in the presence of significantly lower NH_4^+-N concentrations compared to present treatments. For example, ammonium levels did not exceed 180–197 g N m^{-3} in the work performed by Wang et al. (2015). In addition, stable PO_4-P concentrations were used in both studies, while PO_4-P concentration in reactor R_PA gradually increased. Thus, the soluble PO_4-P concentration in R_C achieved 91.6 ± 9.2 mg P L^{-1}, whereas the soluble PO_4-P level in R_PA increased up to 769.2 ± 39.4 mg P L^{-1} on day 90. The detected PO_4-P levels were lower than that of theoretically added (~2132 ± 58 mg P L^{-1} on day 90 in R_PA). On one hand, P plays an important role in microbial growth, fatty acid uptake processes and methane production (Lei et al., 2010; Wang et al., 2015). Therefore, an additional source of P in the system could stimulate the organic acids uptake process with the increase in specific biogas production. On the other hand, a fraction of FAN increases in response to increasing pH, resulting in enhanced effect of toxicity on methanogens (Garcia and Angenent, 2009). Therefore, the presence of H_3PO_4 could reduce the toxic FAN levels in R_PA because of pH decrease and enhance the biogas release from ammonium-rich chicken wastes. Ho and Ho (2012) reported the pH of the piggery wastewater change from 8.3 to 6.5 with concentrated hydrochloric acid correlated with the significant decrease in initial FAN levels and high methane production in batch thermophilic systems.

In stage III, both reactors were functioned at lower HRT of 40 days. Too high phosphate concentrations can inhibit acetogenesis, acidogenesis and methanogenesis (Lei et al., 2010; Wang et al., 2015), and therefore, to avoid phosphate overloading, experimental reactor operated in the absence of H_3PO_4 throughout the remaining experiment. When the HRT was shortened to 40 days, the SBP in R_C had increased up to 426 mL g_{VS}^{-1} (from day 92 till day 110), while, after the period of phosphoric acid addition, the SBP in R_PA had dropped rapidly to 181 mL g_{VS}^{-1} (Fig. 1A). In addition, VFA funneled more effectively into methanogenesis in R_C during this stage, whereas organic acids accumulated in R_PA up to 8.32 g L^{-1} at day 99, indicating insufficient methanogenic activity in the last reactor (Fig. 2A). Therefore, to avoid experimental reactor failure and to investigate the feasibility of its recovery, chicken wastes were not added to R_PA during a 3-day period (days 100–102). After this period, the OLR in R_PA was increased to 1.5 g_{VS} L^{-1} d^{-1} and kept constant until day 110. The next increase of the OLR to 2.3 g_{VS} L^{-1} d^{-1} (days 111–115) successively stabilized the anaerobic process. The received results demonstrated that test reactor had been recovered and finally functioned as well as control reactor (Figs. 1 and 2). The TAN levels were above 4.0 g NH_4^+-N L^{-1}, suggesting that acetate consumption possibly occurred via the syntrophic route under such conditions (Schnürer and Nordberg, 2008). High hydrogen sulfide concentrations detected in both systems might have additionally inhibited methanogenic archaea (Ziganshin et al., 2011). In addition, high H_3PO_4 dose might have increased salinity in R_PA during stage III what might have influenced on microorganisms' activity.

3.2. Bacterial community composition

The bacterial community diversity and shifts in both lab-scale reactors during stages II and III were evaluated using pyrosequencing of 16S rRNA gene amplicons. Table 1 shows the main operating conditions and process parameters of the reactors at three sampling times when microbial community structure was investigated. The quality-checked bacterial 16S rRNA gene sequences amounted to 76,443 in total (Table 2). The composition of the major bacterial phyla with relative abundances greater than 1% in at least one sample is illustrated in Fig. 3A. Collectively the bacterial community in the samples was composed of 11 major and minor phyla as well as candidate divisions WWE1 and WPS-2. Reactor R_C was dominated by *Bacteroidetes* in the first sampling point. However, abundance of *Bacteroidetes* phylotypes precipitously decreased to the end of the experiment (from 49.4% to 4.6% of the pyrotags), and *Firmicutes* phylotypes started to dominate in the second and third samples (71.3% and 89.7% of the pyrotags, respectively). The bacterial community detected in R_PA at day 77 was represented by members of the similar main phyla – *Bacteroidetes* and *Firmicutes*, and the trends towards *Firmicutes* representatives during this reactor operation remained the same.

Fig. 3B demonstrates the taxonomic classification of bacterial reads at genus or higher taxa level comprising at least 1% in at least one sample. Within dominant phylum *Bacteroidetes*, R_C harbored mainly unknown *Porphyromonadaceae* (33.4% of the pyrotags) as well as unclassified *Bacteroidales* (6.4%), *Bacteroidaceae* (5.8%) and *Marinilabiaceae* (2.8%) in the first sample. Compared with the bacterial composition in R_C, *Bacteroidetes* phylum detected in the first sample of R_PA was represented by large numbers of unknown *Bacteroidales* (22.3% of the pyrotags), whereas unknown *Porphyromonadaceae* achieved only 9.5%, *Marinilabiaceae* – 6.4% and *Bacteroidaceae* – 4.8% of the pyrotags. The prevalence of unclassified *Bacteroidales* over unclassified *Porphyromonadaceae* in R_PA compared to R_C might be due to high phosphate loading regime. Species from the *Bacteroidetes* group being acidogenic, saccharolytic, proteolytic bacteria play an important role in anaerobic digestion processes in distinct systems fed with various biomass

Table 1
Operating conditions and process parameters of the lab-scale reactors at three sampling times when microbial community structure was analyzed.

Reactor	Day	OLR (g_{VS} L^{-1} d^{-1})	HRT (day)	Acid capacity (g L^{-1})	Acetate (g L^{-1})	Propionate (g L^{-1})	pH	TAN (g L^{-1})	FAN (mg L^{-1})	SBP (mL g_{VS}^{-1})	SMP (mL g_{VS}^{-1})
R_C	77	2.8	50	3.64 ± 0.13	1.10 ± 0.06	0.41 ± 0.03	7.52	3.39 ± 0.06	250 ± 10.4	284	154
	99	2.8	40	4.81 ± 0.25	1.71 ± 0.11	0.87 ± 0.05	7.78	3.75 ± 0.11	256 ± 8.0	426	252
	127	3.2	40	3.22 ± 0.17	1.05 ± 0.04	0.14 ± 0.01	7.86	4.89 ± 0.10	505 ± 9.8	366	200
R_PA	77	2.8	50	2.81 ± 0.2	1.21 ± 0.12	0.42 ± 0.04	7.39	3.40 ± 0.11	145 ± 5.7	315	158
	99	2.8	40	8.32 ± 0.24	6.59 ± 0.32	1.12 ± 0.08	7.25	3.66 ± 0.08	127 ± 5.0	268	122
	127	3.2	40	2.50 ± 0.1	1.68 ± 0.15	0.57 ± 0.02	7.80	4.17 ± 0.02	372 ± 13	398	231

Standard deviations are shown for VFA, acetate, propionate, TAN and FAN concentrations ($n = 3$).

Table 2
Alpha diversity analysis on reactors' samples.

Domain	Reactor	Sampling time	Number of filtered reads	Observed OTUs	Phylogenetic metrics	Simpson index	Chao 1 index	Shannon index
Bacteria	R_C	Day 77	22,292	207	24.01	0.95	312.79	5.33
	R_C	Day 99	8081	197	24.29	0.93	326.52	5.24
	R_C	Day 127	9502	71	10.20	0.89	133.68	4.17
	R_PA	Day 77	12,993	213	24.21	0.95	285.09	5.45
	R_PA	Day 99	13,438	181	22.25	0.92	294.66	5.09
	R_PA	Day 127	10,137	71	10.47	0.93	91.61	4.64
Archaea	R_C	Day 77	7551	25	3.90	0.42	26.30	1.31
	R_C	Day 99	7353	26	3.02	0.32	30.48	1.08
	R_C	Day 127	7778	24	3.00	0.57	24.80	1.80
	R_PA	Day 77	8118	21	2.90	0.32	25.37	1.08
	R_PA	Day 99	7432	25	3.92	0.41	29.95	1.48
	R_PA	Day 127	7521	25	4.33	0.75	29.45	2.57

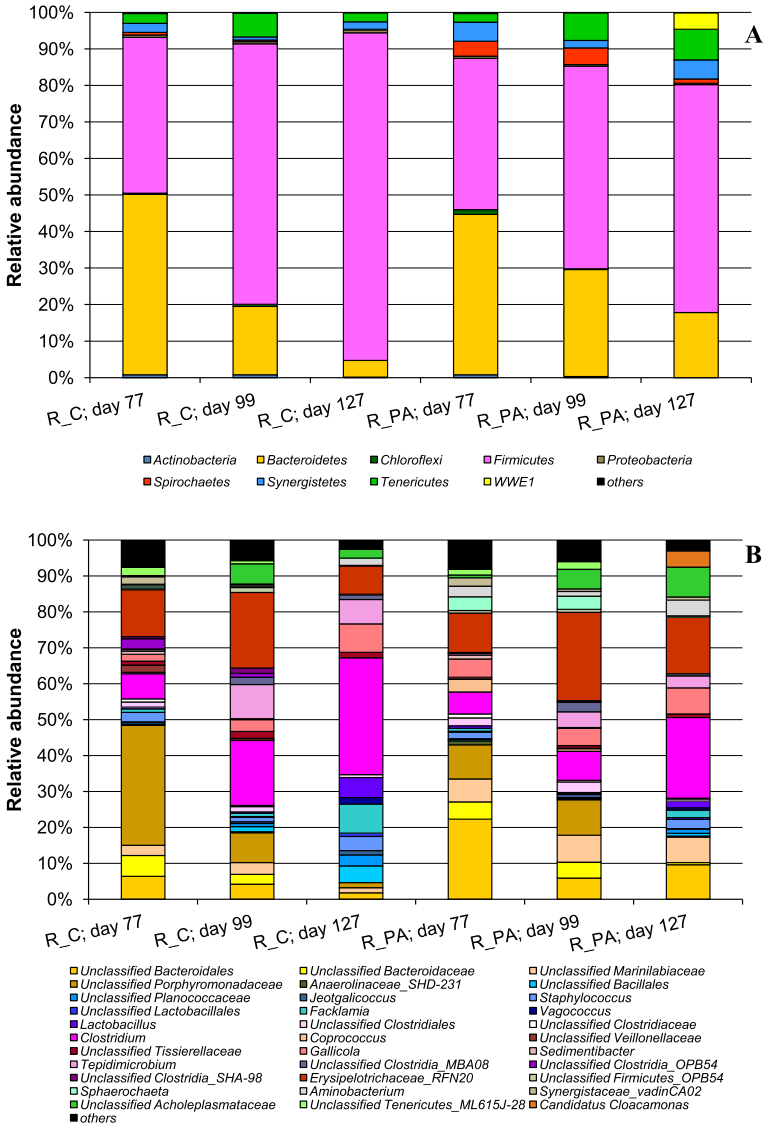

Fig. 3. Relative abundances and dynamics of bacterial taxonomic groups in reactors' samples collected at different operating times. The taxonomic classification of bacterial reads at phylum (A) and genus or higher taxa (B) levels is shown. Bacterial groups accounting for less than 1% of all classified sequences are summarized in the group "others".

(Ziganshin et al., 2011; Ho et al., 2013). The *Bacteroidales* members have been found to be predominant in faeces from humans, ruminants, bovines, horses, suggesting that they are referred to fecal anaerobic bacteria (Tambalo et al., 2012). The reduction in the abundance of all members within *Bacteroidales* order during reactors' operation could be associated with multitude factors, including the increase of OLR, decrease of HRT, high TAN and VFA concentrations, pH change, which all impacted on the activity of *Bacteroidales* group. It is worth noting that dominant representatives could not be classified beyond families within the order

Bacteroidales, limiting the ability to describe their precise functional roles in such treatments.

With increasing OLR from 2.8 to 3.2 $g_{VS}\ L^{-1}\ d^{-1}$ and decreasing HRT from 50 to 40 days, the bacterial phylum *Firmicutes* occupied a dominant position in both reactors. Thus, the abundance of *Firmicutes* members reached 89.7% and 62.4% of the pyrotags in R_C and R_PA at the end of the operating period (day 127), respectively (Fig. 3A). Of this phylum, significant abundance of *Clostridium* sp. was identified in both reactors (up to 32.5% and 22.5% for R_C and R_PA, accordingly) (Fig. 3B). Members of the order *Clostridiales* are represented by hydrolytic bacteria, which are able to ferment sugars to various organic acids and actively produce molecular hydrogen. They have been found in various anaerobic reactor systems (i.e. Ho et al., 2013; Ziganshina et al., 2014b). Since clostridia possess high cellulolytic activity, the impetuous increase of the *Clostridium* phylotype in reactors' samples might be due to the presence of high content of cellulosic biomass (bedding materials) in chicken wastes. Furthermore, representatives of the class *Clostridia* have been found in anaerobic systems, where the bacterial syntrophic acetate oxidation (SAO) is considered to be the primary pathway to control acetate levels. SAO occurs when aceticlastic methanogenesis is inhibited either because of the presence of high ammonia levels (higher than 3.0 $g\ NH_4^+\text{-}N\ L^{-1}$) (Schnürer and Nordberg, 2008) or at high temperatures (Ho et al., 2013). It was previously shown that *Clostridium ultunense* could oxidize acetate in syntrophic association with hydrogenotrophic methanogens (Schnürer et al., 1996). The increase of TAN concentration in both systems was observed (up to ~4.9 $g\ NH_4^+\text{-}N\ L^{-1}$); therefore, SAO pathway with *Clostridium* sp. as the main acetate consumer can be also proposed in both tested reactors. In addition, the proportion of the genera *Gallicola* and *Tepidimicrobium* within the order *Clostridiales* increased in both reactors during the experimental period. *Gallicola barnesae* previously isolated from chicken faeces can produce acetic and lactic acids from peptone/yeast extract/glucose (Ezaki et al., 2001). *Tepidimicrobium xylanilyticum* isolated from the sludge of anaerobic digester treating municipal solid waste and sewage has the ability to grow on various carbohydrates and proteinaceous compounds. *T. xylanilyticum* produces acetate, ethanol, butyrate, hydrogen from glucose, while propionate is produced from xylan (Niu et al., 2009). It can be assumed that the gradual increase of *Clostridiales* (*Clostridium* sp., *Gallicola* sp. and *Tepidimicrobium* sp.) in R_C and R_PA might be additionally associated with their higher tolerance to inhibiting ammonia levels.

It was found that the family *Erysipelotrichaceae* (class *Erysipelotrichi*) became the predominant group in the bacterial amplicon library of the second samples with 21.0% and 24.6% of the pyrotags in R_C and R_PA, respectively; however, lower abundances of this phylotype were detected in both reactors at day 127 (Fig. 3B). Species of this family are found in various livestock manures and comprised of facultatively anaerobic or anaerobic bacteria with chemoorganotrophic fermentative or respiratory metabolism (Ramasamy et al., 2013). Furthermore, a noticeable increase of other representatives of *Firmicutes* in R_C was observed, namely the genera *Facklamia* and *Lactobacillus* within the order *Lactobacillales* (up to 8.1% and 5.6% of the pyrotags), while their relative abundances in R_PA increased insignificantly. Their abundances never exceeded 2% of the pyrotags in R_PA, which functioned in the presence of high phosphate concentrations. Members of the genus *Lactobacillus* are heterofermentative and homofermentative lactic acid bacteria and can be found in various environments where carbohydrates are available (Hammes et al., 2009). *Facklamia* representatives are able to excrete some organic acids from different substrates (Collins et al., 1999). It was also reported that members of *Clostridiales* and *Lactobacillales* were detected as the major taxonomic groups in the intestinal tract of broiler chickens (Lu et al., 2003). Therefore, the gradual increase of *Clostridiales* and *Lactobacillales* in both systems could be additionally associated with the composition of the feedstock. Other phyla were present in minor proportions in both reactors, with the exception of unclassified *Acholeplasmataceae* (phylum *Tenericutes*) and *Aminobacterium* sp. (phylum *Synergistetes*). The relative abundance of *Acholeplasmataceae* increased from undetected levels to 5.6% in reactor R_C at day 99 and to 8.3% of the pyrotags in reactor R_PA at day 127. The relative abundance of *Aminobacterium* sp., which in co-culture with the hydrogenotrophic *Methanobacterium formicicum* oxidizes amino acids (Baena et al., 1998), increased up to 2.0% and 4.4% in reactors R_C and R_PA at day 127, respectively.

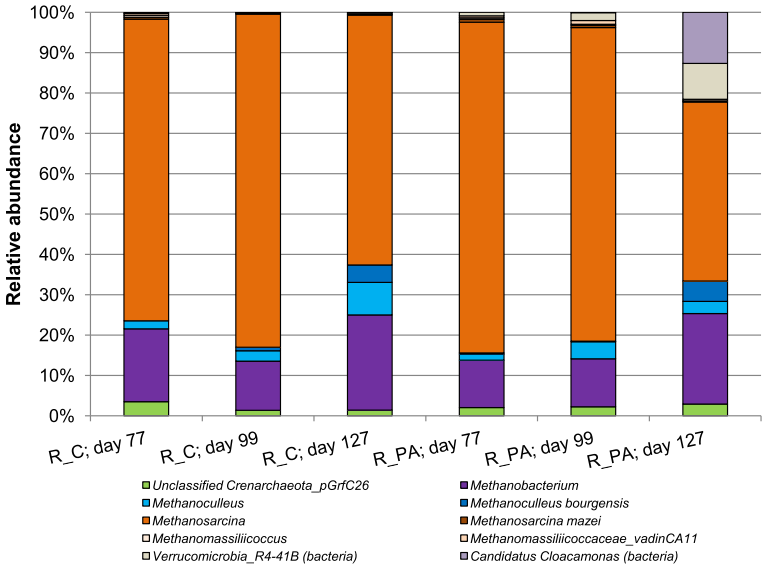

Fig. 4. Relative abundances and dynamics of archaeal taxonomic groups in reactors' samples collected at different operating times. The taxonomic classification of archaeal reads at species or higher taxa levels is shown. In addition to archaeal sequences, a part of the received sequences in R_PA was related to 2 bacterial taxa, mostly in the last sample.

3.3. Archaeal community composition

The archaeal reads obtained using pyrosequencing approach were assigned to 8 phylotypes with *Methanosarcina* as the dominant genus in all analyzed samples. The quality-checked 16S rRNA gene sequences amounted to 45,753 in total (Table 2). Fig. 4 demonstrates the taxonomy of archaeal reads at species or higher taxa level. In addition to archaeal sequences, a part of the received sequences in reactor R_PA was related to 2 bacterial taxa, *Verrucomicrobia* and candidate division WWE1.

Methanosarcina, which is able to perform aceticlastic, hydrogenotrophic and methylotrophic methanogenesis (Demirel and Scherer, 2008), was the most abundant phylotype in the pyrotag dataset and showed the abundance up to 74.6% in R_C and 81.9% in R_PA at day 77 (Fig. 4). *Methanobacterium*, which is a strict hydrogenotrophic genus (Demirel and Scherer, 2008), was the second major phylotype in both systems (18.0% and 11.8% in R_C and R_PA at day 77, respectively). With increasing OLR and decreasing HRT, the proportion of *Methanosarcina* sp. decreased to 61.8% and 44.4% in R_C and R_PA, respectively, but also found to be the dominant phylotype in both reactors at day 127. Furthermore, it was observed the increase of the hydrogenotrophic *Methanobacterium* and *Methanoculleus* in both reactors up to 35.8% in R_C and to 30.4% in R_PA at day 127. The members of *Crenarchaeota* were found to be minor organisms in analyzed samples of both reactors (Fig. 4).

Schnürer and Nordberg (2008) observed a shift to SAO at TAN levels higher than 3 g L^{-1}. Methanogens belonging to *Methanomicrobiales* have been identified as important organisms in SAO as hydrogen-consuming organisms (Schnürer et al., 1999), and methanogens of *Methanosarcinaceae* have also been suggested to be involved in SAO as hydrogen-utilizing organisms (Ho et al., 2013). During both reactors' operation a shift to *Clostridium* sp. was identified, and it was previously reported that e.g. *C. ultunense* could perform the acetate oxidation to H_2 and CO_2 (Schnürer et al., 1996). Based on the present and literature data, it can be assumed that acetate consumption in both systems at high TAN values occurred through SAO with *Clostridium* sp. as the acetate consumer and with *Methanobacterium* sp., *Methanoculleus* sp. and *Methanosarcina* sp. as the hydrogenotrophic partner organisms. However, the involvement of *Methanosarcina* sp. in aceticlastic methanogenesis also cannot be excluded. Therefore, further experiments should be conducted to clarify the precise roles of *Clostridium* sp. and *Methanosarcina* sp. in such systems.

3.4. Correlation of process parameters with microbial community structure and dynamics

Alpha diversity indices were also calculated, including number of observed OTUs, phylogenetic metrics, Simpson index, Chao 1 index, Shannon index, to elucidate the bacterial community diversity in the samples of both reactors (Table 2). In general, the bacterial communities of both reactors were found more diverse in the first and second samples, the phylogenetic distance decreased overtime, the Simpson index was relatively stable, the Chao 1 and Shannon indexes decreased in both systems by the end of the experimental period. Principal coordinate analysis (PCoA) plots of community beta diversity showed the gradual temporal shifts of the bacterial communities during reactors R_C and R_PA operation (Fig. 5A). In accordance with PCoA plot, most of the samples were separately spotted on the PCoA plot without grouping with each other; only two samples collected on days 77 and 99 from R_PA were relatively close to each other. In addition, the NMDS plot calculated from the assigned bacterial reads on the basis of the Bray–Curtis index showed that the samples of reactor R_C differed from the samples of reactor R_PA, and the major process parameter

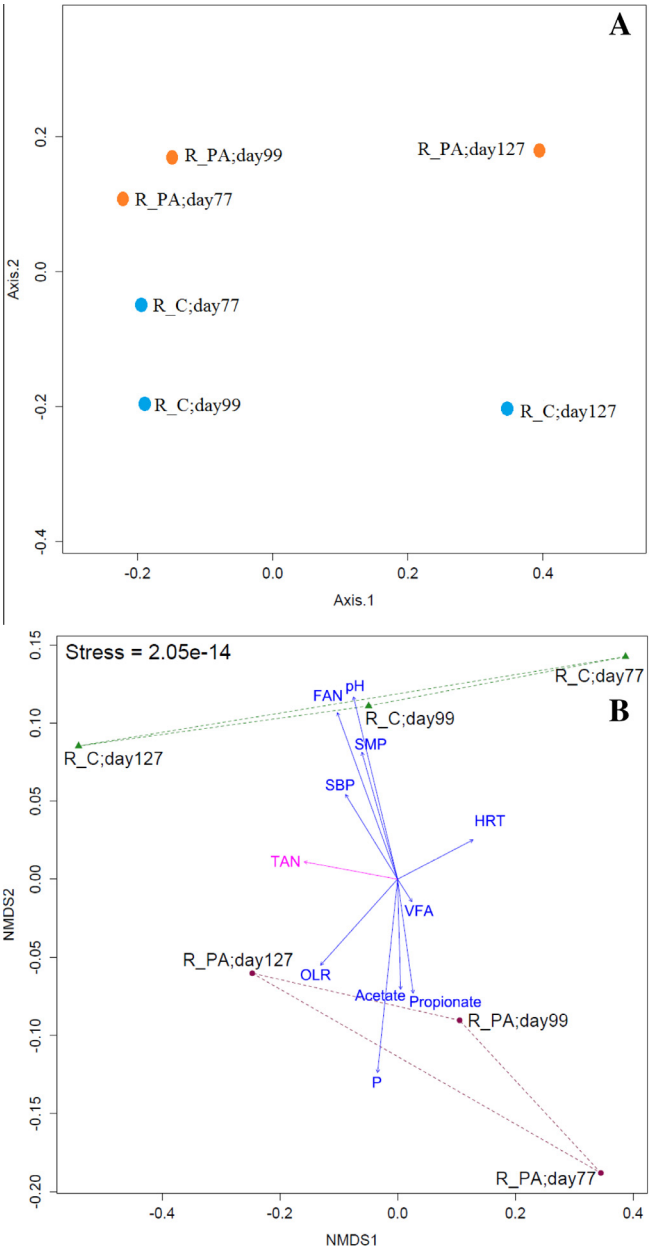

Fig. 5. (A) The beta diversity of bacterial community among two reactors' samples based on PCoA implemented in QIIME. (B) NMDS analysis depicting variations in the bacterial diversity and correlation vectors of community differences and the process parameters across the samples. Magenta arrows indicate the correlation vectors of community differences and the process parameters with significance factors $p < 0.01$. Blue arrows indicate the correlation vectors of community differences and the process parameters with significance factors $0.01 < p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

shaping the bacterial community structure during anaerobic digestion of chicken wastes in both reactors was the high TAN level ($p < 0.01$) (Fig. 5B). Correlation analysis also revealed that, besides the TAN concentration, the presence of high phosphate, acetate, propionate concentrations and OLR variations ($p < 0.05$) led to the development of distinct bacterial community in R_PA. Thus, the results demonstrated that the bacterial community structure changed during reactors' operation.

The temporary changes of archaeal community in both reactors were also assessed using standard biodiversity indices. In general, the communities of both reactors were found to be stable in all

samples, the phylogenetic distance decreased overtime in R_C but increased in R_PA, the Simpson index increased overtime, the Chao 1 index varied insignificantly, and Shannon index increased in both systems by the end of the operating period (Table 2). PCoA plots demonstrated the gradual temporal shifts of the archaeal communities during R_C and R_PA operation (Fig. 6A). In accordance with PCoA plot, only three samples collected on day 77 from R_PA and on days 99 and 127 from R_C were relatively close to each other. In addition, the NMDS plot showed that four samples of R_C and R_PA grouped to each other, while the last two samples of R_C and R_PA differed from each other (Fig. 6B). The major process parameters shaping the archaeal community structure in R_C were the high TAN and FAN levels ($p < 0.01$) as well as the OLR and pH values ($p < 0.05$). The archaeal community structure in R_PA was also influenced by high phosphate level ($p < 0.05$). Thus, the received results illustrated that the archaeal communities changed throughout reactors' operation.

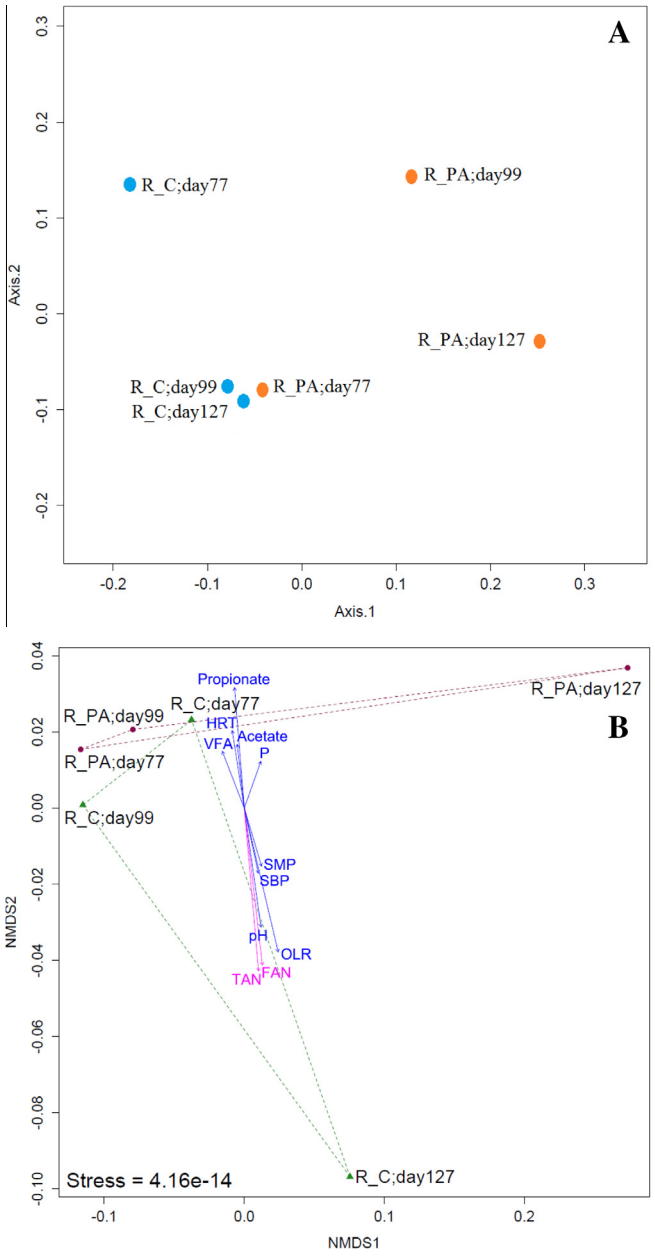

Fig. 6. (A) The beta diversity of archaeal community (including two bacterial phylotypes) among two reactors' samples based on PCoA implemented in QIIME. (B) NMDS analysis depicting variations in microbial diversity and correlation vectors of community differences and the process parameters across the samples. Magenta arrows indicate the correlation vectors of community differences and the process parameters with significance factors $p < 0.01$. Blue arrows indicate the correlation vectors of community differences and the process parameters with significance factors $0.01 < p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

The increasing OLR and decreasing HRT influenced on performance of reactors stabilized on chicken wastes. Additionally, experiments indicated that organic acids uptake process and their conversion into methane were accelerated by moderate phosphoric acid concentrations. The major process parameter that influenced on the microbial community structure in both reactors was the high ammonium concentration. During reactors' operation, *Bacteroidetes* phylotypes were replaced with *Firmicutes* phylotypes, while *Methanosarcina* sp. dominated in all analyzed samples, in which high TAN levels were detected. These results can help in better understanding the anaerobic digestion process of simultaneously ammonium- and phosphate-rich substrates.

Conflict of interest

All authors declare that they have no conflict of interest.

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References

Abouelenien, F., Fujiwara, W., Namba, Y., Kosseva, M., 2010. Improved methane fermentation of chicken manure via ammonia removal by biogas recycle. Bioresour. Technol. 10, 6368–6373.

Baena, S., Fardeau, M.L., Labat, M., Ollivier, B., Thomas, P., Garcia, J.L., Patel, B.K., 1998. *Aminobacterium colombiensegen*. nov. sp. nov., an amino acid-degrading anaerobe isolated from anaerobic sludge. Anaerobe 4, 241–250.

Collins, M.D., Hutson, R.A., Falsen, E., Sjödén, B., 1999. *Facklamia sourekii* sp. nov., isolated from human sources. Int. J. Syst. Bacteriol. 49, 635–638.

Costa, J.C., Barbosa, S.G., Alves, M.M., Sousa, D.Z., 2012. Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. Bioresour. Technol. 11, 141–147.

Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol. 7, 173–190.

Ezaki, T., Kawamura, Y., Li, N., Li, Z.Y., Zhao, L., Shu, S., 2001. Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. Int. J. Syst. Evol. Microbiol. 51, 1521–1528.

Fotidis, I.A., Karakashev, D., Kotsopoulos, T.A., Martzopoulos, G.G., Angelidaki, I., 2013. Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. FEMS Microbiol. Ecol. 83, 38–48.

Fotidis, I.A., Karakashev, D., Angelidaki, I., 2014. The dominant acetate degradation pathway/methanogenic composition in full-scale anaerobic digesters operating under different ammonia levels. Int. J. Environ. Sci. Technol. 11, 2087–2094.

Garcia, M.L., Angenent, L.T., 2009. Interaction between temperature and ammonia in mesophilic digesters for animal waste treatment. Water Res. 43, 2373–2382.

Hammes, W.P., Hertel, C., 2009. Genus I. Lactobacillus Beijerink, 1901. In: De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, Berlin.

Ho, L., Ho, G., 2012. Mitigating ammonia inhibition of thermophilic anaerobic treatment of digested piggery wastewater: use of pH reduction, zeolite, biomass and humic acid. Water Res. 46, 4339–4350.
Ho, D.P., Jensen, P.D., Batstone, D.J., 2013. *Methanosarcinaceae* and acetate-oxidizing pathways dominate in high-rate thermophilic anaerobic digestion of waste-activated sludge. Appl. Environ. Microbiol. 79, 6491–6500.
Lei, Z., Chen, J., Zhang, Z., Sugiura, N., 2010. Methane production from rice straw with acclimated anaerobic sludge: effect of phosphate supplementation. Bioresour. Technol. 101, 4343–4348.
Li, Y., Zhang, R., He, Y., Zhang, C., Liu, X., Chen, C., Liu, G., 2014. Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). Bioresour. Technol. 156, 342–347.
Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69, 6816–6824.
Lv, Z., Hu, M., Harms, H., Hermann, H., Richnow, H., Liebetrau, J., Nikolausz, M., 2014. Stable isotope composition of biogas allows early warning of complete process failure as a result of ammonia inhibition in anaerobic digesters. Bioresour. Technol. 167, 251–259.
Nie, H., Jacobi, F., Strach, K., Xu, C., Zhou, H., Liebetrau, J., 2015. Mono-fermentation of chicken manure: ammonia inhibition and recirculation of the digestate. Bioresour. Technol. 178, 238–246.
Nielsen, H.B., Angelidaki, I., 2008. Strategies for optimizing recovery of the biogas process following ammonia inhibition. Bioresour. Technol. 99, 7995–8001.
Niu, L., Song, L., Liu, X., Dong, X., 2009. *Tepidimicrobium xylanilyticum* sp. nov., an anaerobic xylanolytic bacterium, and emended description of the genus *Tepidimicrobium*. Int. J. Syst. Evol. Microbiol. 59, 2698–2701.
Niu, Q., Qiao, W., Qiang, H., Hojo, T., Li, Y.-Y., 2013a. Mesophilic methane fermentation of chicken manure at a wide range of ammonia concentration: stability, inhibition and recovery. Bioresour. Technol. 137, 358–367.
Niu, Q., Qiao, W., Qiang, H., Li, Y.-Y., 2013b. Microbial community shifts and biogas conversion computation during steady, inhibited and recovered stages of thermophilic methane fermentation on chicken manure with a wide variation of ammonia. Bioresour. Technol. 146, 223–233.
Ramasamy, D., Lagier, J.C., Nguyen, T.T., Raoult, D., Fournier, P.E., 2013. Non contiguous-finished genome sequence and description of *Dielma fastidiosa* gen. nov., sp. nov., a new member of the family *Erysipelotrichaceae*. Stand. Genomic Sci. 8, 336–351.
Schmidt, T., Ziganshin, A.M., Nikolausz, M., Scholwin, F., Nelles, M., Kleinsteuber, S., Pröter, J., 2014. Effects of the reduction of the hydraulic retention time to 1.5 days at constant organic loading in CSTR, ASBR, and fixed-bed reactors – performance and methanogenic community composition. Biomass Bioenergy 69, 241–248.
Schnürer, A., Nordberg, A., 2008. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci. Technol. 57, 735–740.
Schnürer, A., Schink, B., Svensson, B.H., 1996. *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. Int. J. Syst. Bacteriol. 46, 1145–1152.
Schnürer, A., Zellner, G., Svensson, B.H., 1999. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. FEMS Microbiol. Ecol. 29, 249–261.
Tambalo, D.D., Fremaux, B., Boa, T., Yost, C.K., 2012. Persistence of host-associated *Bacteroidales* gene markers and their quantitative detection in an urban and agricultural mixed prairie watershed. Water Res. 46, 2891–2904.
Wang, Q., Yang, Y., Yu, C., Huang, H., Kim, M., Feng, C., Zhang, Z., 2011. Study on a fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes. Bioresour. Technol. 102, 7064–7068.
Wang, X., Yang, G., Feng, Y., Ren, G., Han, X., 2012. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour. Technol. 120, 78–83.
Wang, R., Li, Y., Wang, W., Chen, Y., Vanrolleghem, P.A., 2015. Effect of high orthophosphate concentration on mesophilic anaerobic sludge digestion and its modeling. Chem. Eng. J. 260, 791–800.
Ziganshin, A.M., Gerlach, R., Naumenko, E.A., Naumova, R.P., 2010. Aerobic degradation of 2,4,6-trinitrotoluene by the yeast strain *Geotrichum candidum* AN-Z4. Microbiology 79, 178–183.
Ziganshin, A.M., Schmidt, T., Scholwin, F., Il'inskaya, O.N., Harms, H., Kleinsteuber, S., 2011. Bacteria and archaea involved in anaerobic digestion of distillers grains with solubles. Appl. Microbiol. Biotechnol. 89, 2039–2052.
Ziganshin, A.M., Ziganshina, E.E., Pröter, J., Kleinsteuber, S., Il'inskaya, O.N., 2012. Methanogenic community dynamics during anaerobic utilization of agricultural wastes. Acta Nat. 4, 91–97.
Ziganshina, E.E., Belostotskiy, D.E., Shushlyaev, R.V., Miluykov, V.A., Vankov, P.Y., Ziganshin, A.M., 2014a. Microbial community diversity in anaerobic reactors digesting turkey, chicken, and swine wastes. J. Microbiol. Biotechnol. 24, 1464–1472.
Ziganshina, E.E., Bagmanova, A.R., Khilyas, I.V., Ziganshin, A.M., 2014b. Assessment of a biogas-generating microbial community diversity in a pilot-scale anaerobic reactor. J. Biosci. Bioeng. 117, 730–736.