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Acclimatization contributes to stable anaerobic digestion of organic fraction of municipal solid waste under extreme ammonia levels: Focusing on microbial community dynamics

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Abstract

The organic fraction of municipal solid waste (OFMSW) is an abundant and sustainable substrate for the anaerobic digestion (AD) process, yet ammonia released during OFMSW hydrolysis could result in suboptimal biogas production. Acclimatized ammonia tolerant microorganisms offer an efficient way to alleviate ammonia inhibition during AD. This study aimed to achieve an efficient AD of OFMSW under extreme ammonia levels and elucidate the dynamics of the acclimatized microbial community. Thus, two mesophilic continuous stirred tank reactors (CSTR), fed only with OFMSW, were successfully acclimatized up to 8.5 g NH_4^+-N/L, and their methane yields fluctuated less than 10%, compared to the methane yields without ammonia addition. Microbiological analyses showed that *Methanosaeta concilii* and *Methanosarcina soligelidi* were the dominant methanogens at low and high ammonia levels, respectively. Whilst, a unique metabolic pathway shift, from aceticlastic to hydrogenotrophic methanogenesis, of *M. soligelidi* was identified during the acclimatization process.

Keywords

Ammonia acclimatization; Hydrogenotrophic pathway; Aceticlastic pathway; metabolic pathway shift; *Methanosarcina soligelidi*

1. Introduction

The increasing global need for natural resources and consumer goods leads to vast amounts of municipal solid waste (MSW). In 2018, more than 2.5 Mt of MSW were generated in the European Union (Eurostat, 2018), with 46% of them been organic (OFMSW: organic fraction of MSW) (Hoornweg and Bhada-Tata, 2012). Improper treatment methods of OFMSW can cause environmental and health issues (Fisher, 2006). Currently, most of the MSW is treated in one of the four ways: landfilling (24%), incineration (28%), recycling (29%), and composting (16%) (Eurostat, 2018). Landfilling and incineration methods do not take advantage of the organic faction and the nutrients of OFMSW and can lead to extra greenhouse gas emissions (Fisher, 2006; Eurostat, 2018). At the same time, huge consumption and shortage of fossil fuels motivate researchers to find alternative energy sources. Thus, new treatment technologies must be developed to not only offset the use of fossil fuels but also maximize the reuse of the vast amounts of the OFMSW and thereby maintain and recycle the useful nutrient back to agriculture.

Anaerobic digestion (AD) is a process that can convert organic waste into sustainable energy (biogas), via a series of interrelated microbial metabolisms (Campanaro et al., 2018). The AD process is divided in four steps (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis), which are mediated primarily by bacteria and archaea (Angelidaki et al., 2011). In addition, the liquid product of the AD process (digestate) contains high levels of nitrogen and phosphorus, which can be potentially reused as biofertilizer or after extraction, as supplement for fermentation processes. In spite of these benefits, the degradation of OFMSW produces ammonia, a by-product from the catabolism of proteins, which can be toxic to the AD process, resulting in poor operational stability and reduced methane production efficiency (Tian et al., 2018b).

Total ammonia (TAN) is the sum of ammonium ions (NH_4^+) and free ammonia (FAN, NH_3), while FAN exists in an equilibrium defined by the pH and the temperature (Tian et al., 2017). As Koster and Lettinga (1988) reported, when ammonia concentrations ranged from 4 to 5.7 g NH_4^+-N/L, more than 56% of the methanogenic activity was inhibited in a granular sludge reactor. The reason is that FAN is freely membrane-permeable and hence, decreases methanogenic activity by interfering with the natural intracellular biological pathways of the methanogens (e.g. inhibiting specific methane synthesizing enzyme reaction) (Sprott and Patel, 1986). It is generally accepted that acetoclastic methanogens are more sensitive to ammonia toxicity compared to hydrogenotrophic methanogens (Schnürer and Nordberg, 2008). For example, hydrogenotrophic methanogenic pathway (coupled with syntrophic acetate oxidation) became dominant (by replacing the acetoclastic pathway) in a semi-continuous lab-scale anaerobic digester, when ammonia exceeded 3 NH_4^+-N g/L (Wiegant and Zeeman, 1986). However, there is conflicting information in the literature about the sensitivity of acetoclastic methanogens to ammonia, with few researchers reporting that different members of the acetoclastic *Methanosarcina* spp. could develop tolerance to ammonia toxicity.

Overall, the ammonia inhibition levels are case sensitive and are defined by the nature of substrate, the inoculum, the reactor type, the operating parameters (pH and temperature), the mixing and the acclimatization period. For example, 50% inhibition was observed at 3.9 and 5.6 g NH_4^+-N/L in mesophilic and thermophilic batch reactors, respectively that were digesting OFMSW (Liu et al., 2015). While another study found that 1 g NH_4^+-N/L in MSW's leachate was enough to inhibit the methane production with 25% loss in a mesophilic expanded granular sludge bed reactor (Nielsen and Angelidaki, 2008). Stepwise acclimatization to high ammonia levels is a common method used to

increase ammonia tolerance in the AD microbiome (Akindele and Sartaj, 2018; Tian et al., 2018a). In such a process, mesophilic methanogenic consortia were successfully acclimatized to 5 g NH_4^+-N/L (at pH 7.50) in batch reactors, with OFMSW as substrate (Tian et al., 2018b). However, acclimatization in continuous reactors to overcome ammonia inhibition and improve methane production efficiency of OFMSW has never been reported.

Therefore, the preliminary aim of the present study was to achieve an efficient and stable continuous anaerobic degradation of OFMSW under extreme ammonia levels (>7 g NH_4^+-N/L). While, the main aim of the study was to elucidate the dynamics of the microbial community under different ammonia levels. To realize these aims, two continuous stirred tank reactors (CSTR) were fed with OFMSW, while a stepwise ammonia acclimatization process was followed.

2. Material and methods

2.1. Inoculum and feedstock

The inoculum used to start up the two CSTR reactors originated from the mesophilic (37°C) anaerobic digesters, which run for 4 months fed with OFMSW. The substrate used in this study was OFMSW treated with the biopulping process to increase the biodegradability by Gemidan Ecogi A/S. The substrate was kept at -20ºC until use, when thawed at room temperature and diluted with distilled water to a fixed TS content of 60 g VS/kg. The characteristics of the inoculum and the biopulp are presented in Table 1.

Table 1. Characteristics of inoculum and substrate

Parameter	Unit	Inoculum (SD)	Biopulp (SD)

Total solids (TS)	g/kg	38.61 (0.34)	60.52 (0.52)
Volatile solids (VS)	g/kg	24.07 (0.35)	52.11 (0.55)
pH	-	8.38	3.90
Total ammonia nitrogen (TAN)	g NH_4^+-N/L	3.80 (0.01)	0.40 (0.01)
Free ammonia nitrogen (FAN)	g NH_3-N/L	0.896 (0.01)	0.004 (0.01)
Total Kjeldahl Nitrogen (TKN)	g N/L	5.38 (0.25)	1.70 (0.03)

* SD: standard deviation.

2.2. Experimental setup

Two CSTR reactors (i.e. R1 and R2), with total and working volume of 4.5 and 3 L, respectively, were fed with OFMSW for 159 days with an organic loading rate of 2.5 g VS/L/day where timer was used to control feeding peristaltic pump to inject 75ml OFMSW per 12 hours. Each reactor was equipped with an influent and an effluent bottle, and a feeding peristaltic pump. Electrical heating jackets maintained the reactors' temperature at 37°C and intermittent stirring with the rate of 30 s/min was applied for mixing the reactor content (worked 34 sec every 56 sec). Water-displacement gas meters were used to measure biogas production. The TAN levels of the influent and the reactor content were increased stepwise from 1.1 to 9.5 g NH_4^+-N/L with the addition of urea ($CO(NH_2)_2$) and ammonium chloride (NH_4Cl). To be more specific, every increase of TAN (1 - 1.5 g NH_4^+-N/L^{-1} each step) both inside the reactor and the feedstock in the influent bottle was implemented when there were no significant decrease (more than 85%

of methane yield during the baseline period (P1)). The whole experiment was divided into seven phases as presented in Table 2.

Table2. The CSTR reactors experimental design

Phase	Days	TAN	Extra added ammonia	
			$CO(NH_2)_2$	NH_4Cl
		(g NH_4^+-N/L)	(g NH_4^+-N/L)	(g NH_4^+-N/L)
P1	0-30	1.1	0	0
P2	31-51	4	1	1.9
P3	52-66	5	2	1.9
P4	67-74	6	2	2.9
P5	75-89	7	3	2.9
P6	90-127	8.5	4	3.4
P7	128-159	9.5	4.5	3.9

2.3. Analyses

2.3.1. Chemical analyses

Methane concentration was measured with the gas chromatography as described by APHA (2005). VFA levels were analysed with gas chromatograph (TRACE 1300 from Thermo Scientific) equipped with flame ionization detector and a DB-FFAP fused silica capillary column. PHM99 LAB pH meter (RadiometerTM) was used to measure the pH. TS, VS, TAN and TKN, were determined according to Angelidaki and Ahring (1993a).

2.3.2. Microbial analysis

Five triplicate samples were taken from both reactors on days 33 (P1), 55 (P2), 77 (P3), 92 (P5) and 162 (P7) and analysed using 16S rRNA gene sequencing technique to elucidate the microbial community shift. After cleaning step with Phenol:Chloroform: Isoamyl Alcohol (25: 24: 1), genomic DNA were extracted using DNeasy PowerSoil® (QIAGEN GmbH, Hilden, Germany). PCR amplification using universal primers 515F/806R was performed on the V4 region of 16S rRNA gene and the amplicons were sequenced using MiSeq desktop sequencer at Ramaciotti Centre for Genomics (Sydney, Australia. Raw reads were deposited in Sequence Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra) with the name of SUB5320995.

The raw data were processed using CLC Workbench software (11.0.1) with the Microbial genomics module plugin. A trimming procedure was used to low-quality reads according to default parameters provided by the software and paired sequences were merged. Chimera crossover filter was also applied. Operational taxonomic units' (OTUs) clustering percent was set at 97%, with threshold for de novo OTUs creation set at 80% and minimum reads occurrence at five. Alpha diversity was calculated based on the number of OTUs and Chao 1 bias-corrected. Beta diversity (Principal Component Analysis) was calculated by Bray-Curtis matrix.

2.4. Calculations and statistics

2.4.1. Free ammonia

Free ammonia concertation was calculated with Eq. (1):

$$FAN=TAN/(1+10^{-pH}/K_a) \qquad \text{Eq. (2)}$$

Where K_a is the dissociation constant with the value of 1.29×10^{-9} at 37°C.

2.4.2. Statistical analysis

All statistics and figures were performed using the Origin software (OriginLab Corporation, USA). Descriptive statistics were carried out for all variables, mean values and standard deviations were calculated. Pearson correlation and hierarchical clustering analyses were carried out using Multiexperiment viewer (MeV4.9.0).

3. Results and discussion

3.1. Reactors' performance

P1, where an average methane yield of 345 ± 40 and 391 ± 59 mL CH_4/g VS was observed for R1 and R2, respectively, was used as baseline phase (no ammonia addition) to evaluate the reactors' performance in the following ammonia acclimatization phases (P2-P7). During P2 to P5, both reactors experienced inhibition with an average loss of 15% of the methane yield compared to P1 (Fig. 1a). While at P6 (8.5 g NH_4^+-N/L) the methane production of both reactors, recovered with methane yields constantly above 94% compared to P1. Specifically, during P6 the average methane production yields did not change significantly and were 332 ± 37 and 369 ± 41 mL CH_4/g VS for R1 and R2, respectively. However, when ammonia was increased to 9.5 g NH_4^+-N/L (P7), both reactors' yields were reduced by more than 15% and the AD process operated under an "inhibited steady state". This stable but suboptimal steady-state is known as an ammonia induced "inhibited steady-state" and is a typical characteristic of many full-scale reactors that operate under high ammonia levels (Benabdallah El Hadj et al., 2009). Even though, there was a clear reduction of methane production at P7, it was significantly less than

expected compared to previous studies. For example, Moestedt et al. (2016) have reported a 50% decrease could be observed in methane production of a mesophilic reactor when TAN exceeded 3.9 g NH^{+}_{4}-N/L.

The VFA accumulation in the two reactors was evolved mostly as expected, based on the methane production (Fig. 1b). Specifically, the VFA levels raised up to 4000 mg HAc/L at the end of P2, but decreased rapidly at the beginning of P3 due to the acclimatization (Tian et al., 2018a) and remained at low levels until P6. Stepwise ammonia acclimatization has been demonstrated before in co-digestions of manure and protein-rich substrates such as microalgae (Angelidaki and Ahring, 1993a). However, this was the first time that continuous AD reactors could acclimatize to this extreme ammonia levels (7 g NH_4^+-N/L) while mono-digesting the OFMSW.

During P6, despite the fact that methane production was stable and above 94% compared to P1, the VFA levels increased and remained above the defined threshold of 1500 mg HAc/L for a “healthy” AD process in CSTR reactors (De Vrieze et al., 2017). It appears that the 8.5 g NH_4^+-N/L (P6) was the threshold to the acclimatization process, which could be attributed to the FAN attained at this period (> 800 mg NH_3-N/L and 8.0 ±0.2, respectively, Fig. 2a). It is generally accepted that FAN is the most toxic form of ammonia, which has positive correlation with pH (Fig. 2b) (Angelidaki and Ahring, 1993b). Thus, despite that both reactors were acclimatised to more than tenfold higher FAN levels (P1 to P5, <50 to >500 mg NH_3-N/L, respectively), FAN concentrations above 700 mg NH_3-N/L were found to be very toxic for AD processes (Xu et al., 2014).

Finally, the VFA accumulation during P7 was severe and both reactors exceeded 4000 mg HAc/L, which is a clear indicator of an AD process failure (Lv et al., 2018). Interestingly, even at these extremely high ammonia levels, the acclimatized microbiome

of the reactors was able to perform at high efficiency levels (methane production around 85% compared to P1).

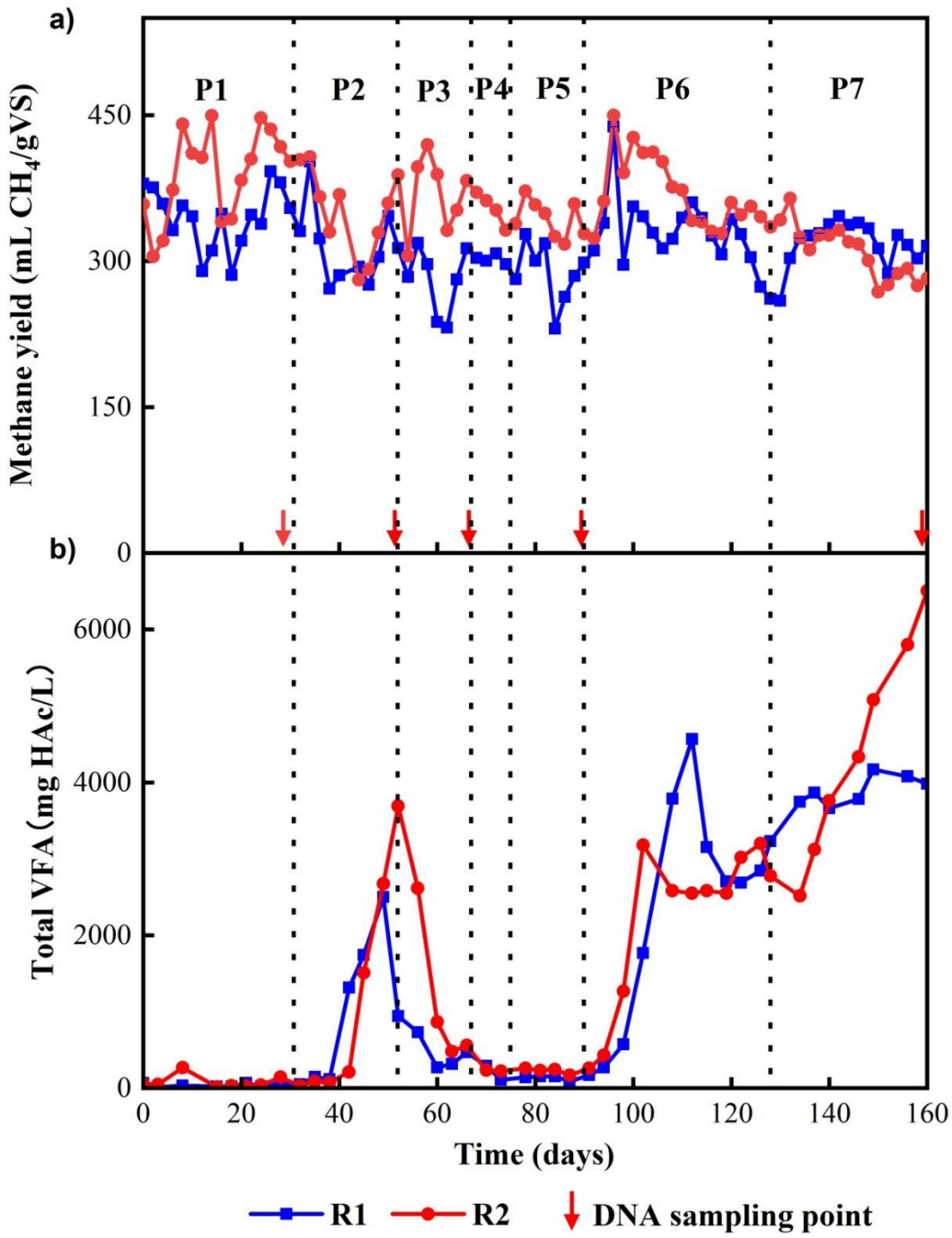

Figure 1. a) CH_4 production, and b) VFA variation throughout the experimental period.

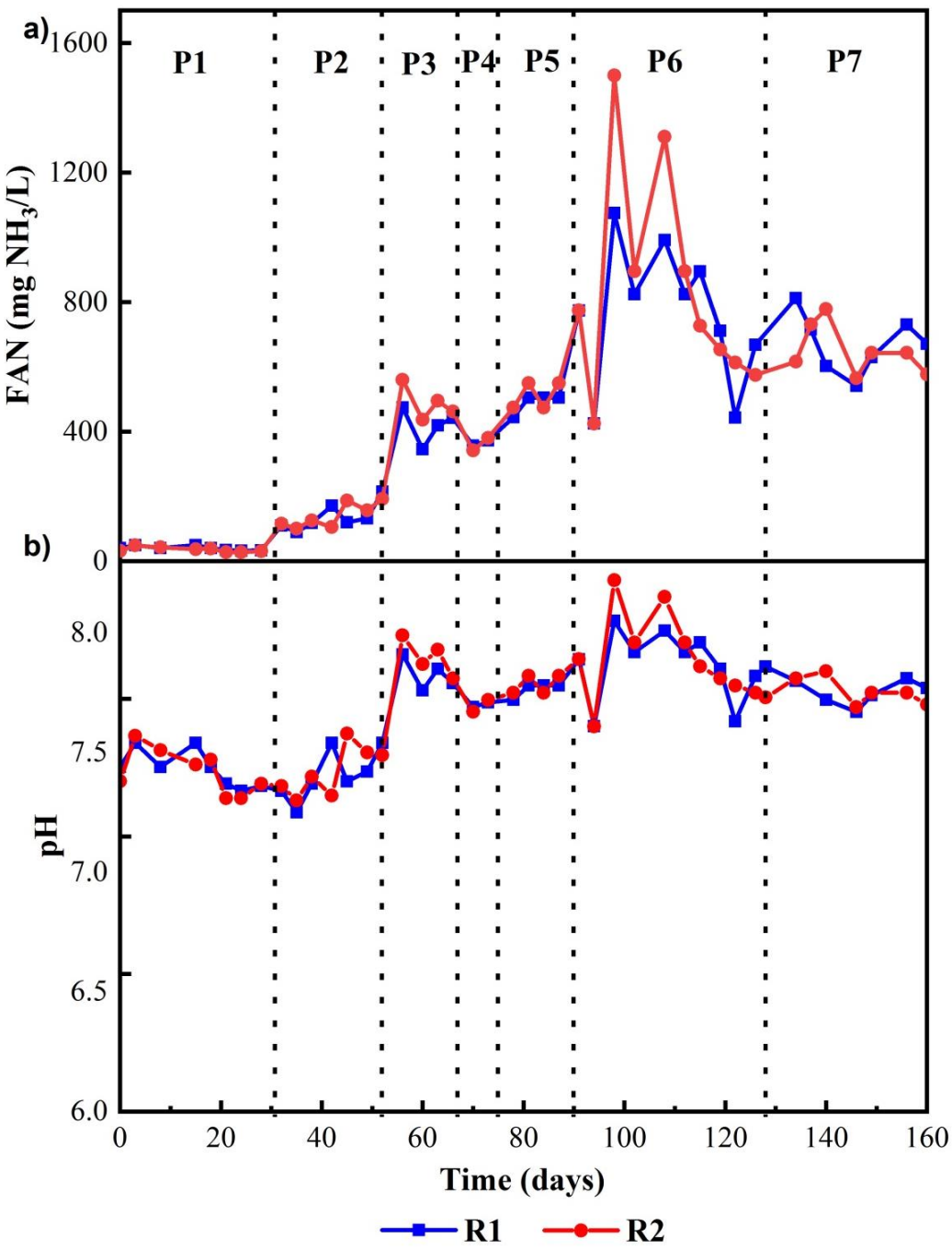

Figure 2. a) FAN, b) pH throughout the experimental period.

3.2. Global microbial dynamics

16S rRNA sequencing was used to elucidate the responses of the microbial community to the increased ammonia concentrations. Alpha diversity based on Chao 1 bias-corrected index (Fig. 3a) showed slight increase at P2 and stable trend alongside the increase of ammonia levels until P6. Afterwards, a sharp decrease of their alpha diversity was observed at P7, which indicated that ammonia increase from 8.5 to 9.5 g NH_4^+-N/L, was enough to washout approximately 12.5% of the microbial species from the reactors based on Chao 1 bias-corrected index. At the same time, Beta diversity (Fig. 3b) showed a clear microbial dynamic trend throughout the experimental process. Specifically, longest matrix distances were found between P1 and P7 for both reactors, followed by the one between P6 and P7. This indicated that when ammonia concentration exceeded 8.5 g NH_4^+-N/L, it drove the microbiome clustering shift (De Vrieze et al., 2017). According to De Vrieze (2017), high diversity and redundancy of microbial community (i.e. as detected from P1 to P6) was considered an effective strategy as buffer against ammonia inhibition, ensuring continuation of methane production, maintaining functionality or metabolic flexibility (Steinhaus et al., 2007). The sudden decrease of alpha diversity from P6 to P7 could have contributed rendering the reactors to become vulnerable to ammonia toxicity. Inhibition was confirmed by the accumulation of VFA (> 4000 mg HAc/L) and decrease of methane production (<312 mL CH_4/g VS) during P7. Therefore it is clear that the toxicity threshold of the two CSTR reactors, based on production performance and microbial activity, for this specific ammonia acclimatization process was 8.5 g NH_4^+-N/L.

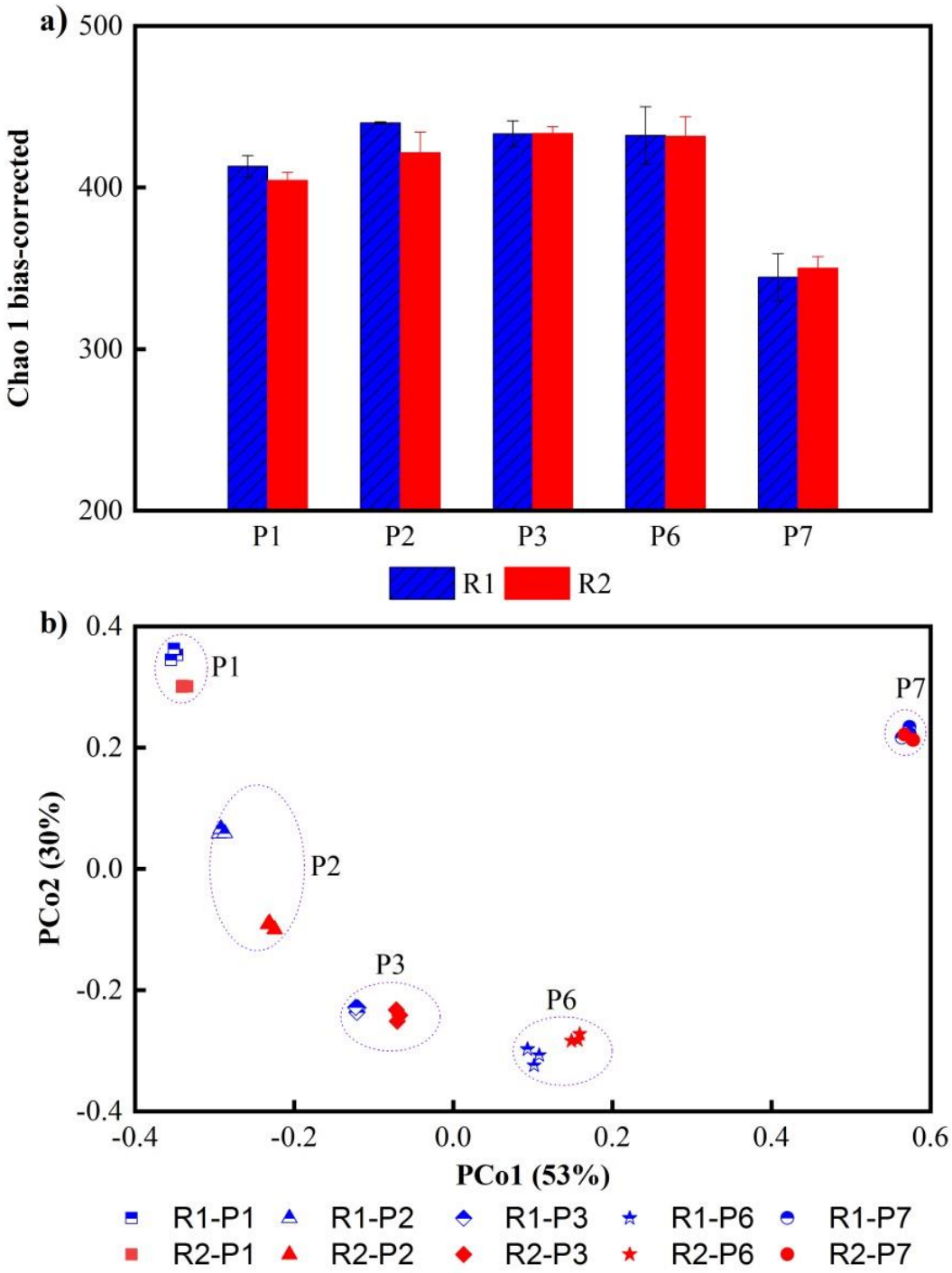

Figure 3. Alpha Diversity a) based on Chao 1 bias-corrected index; b) Beta diversity of the triplicate samples in R1 and R2. Principal components (PC) 1 and 2 explained 53% and 30% of community variation, respectively.

3.2.1. Shift in archaea dominance

Among the archaea, two interesting methanogens changed significantly its abundance from P1 to P7 (Fig. 4), indicating different tolerance capability in respect to ammonia. At P1, the main methanogen was *Methanosaeta concilii* 2, as a specialist species that uses only acetate, which accounted for more than 70% of total archaea population in both reactors (Fig. 4). Considering its abundance levels (still higher than 50% during P2), the dominant role in the archaeal community was well pronounced at medium-low levels of ammonia (< 5 g NH_4^+-N/L). During P3-7, the relative abundance of *M. concilii* 2 decreased alongside the increasing ammonia levels, dropping to 21% (P3), 5% (P6) and 0% (P7). It is explained by two reasons: on the one hand, *M. concilii* 2 can use acetate at concentration levels at the range of 5-20 μM, however acetate level at P6 was far higher than the optimum range. On the other hand, *M. concilii* 2 was found to be the most ammonia-sensitive methanogen, which was completely inhibited at 0.56 g NH_4^+-N/L, primarily due to ammonia's effect on intracellular ion exchange (Ince et al., 2011). Hence, results indicated that there is a negative impact of increasing acetate and ammonia concentrations on *M. concilii* 2 (Tian et al., 2018a).

Conversely, the relative abundance of *Methanosarcina soligelidi* 1 strongly increased from less than 0.4% up to more than 94% in both reactors from P1 to P7. Remarkably, *M. soligelidi* 1 was found to be the most ammonia tolerant methanogen in this study. This finding was in accordance with a previous study, where *M. soligelidi* 1 was the dominant acetoclastic methanogens at extreme ammonia levels (> 7 g NH_4^+-N/L) (Taubner et al., 2015). The dominant role of *M. soligelidi* 1 could be justified by several factors. Firstly, it is known that *M. soligelidi* 1 could form clusters of cells in order to reduce the toxicity of ammonia since this formation creates an ammonium gradient from the bulk ammonia

concentration at the surface along the inner part of the aggregate and thereby lessen the ammonia concentration at the inner core of the sphere (Conklin et al., 2006; Wagner et al., 2013). Secondly, *M. soligelidi* 1 show remarkable capability of environmental stress tolerance (e.g. freezing, aerobic or dry condition) owning to its intact membrane lipids (Wagner et al., 2013). Thirdly, *M. soligelidi* 1 is metabolically more versatile (i.e. able to generate CH_4 from H_2/CO_2, methanol and acetate) than other acetoclastic and hydrogenotrophic methanogens (who are restricted with only carbon substrate) (Li et al., 2017). Additionally, despite the fact that, in many cases, *Methanosarcina* spp. were found to be more sensitive to ammonia compared to hydrogenotrophic methanogens (Wang et al., 2015; Li et al., 2017; Akindele and Sartaj, 2018); in this study, *M. soligelidi* 1 was able to become the dominant methanogen. Conversely, the hydrogenotrophic methanogens were found at extremely low abundance. Specifically, the relative abundance of *Methanobacterium* sp. 3 accounted for 7% at P1 increased to 18% at P6, but completely disappeared at P7, which was similar to the relative abundance of *Methanobacteriaceae* sp. 5. *Methanoculleus palmolei* 4 was the only hydrogenotrophic methanogen present at P7, although at low relative abundance (2% of archaeal community).

Considering the dominant ammonia tolerant methanogens are different in previous studies, it seems that dominant methanogens could be different at the same ammonia levels due to the different operating conditions (e.g. substrate, HRT, OLR, pH etc.) (Wang et al., 2015; Akindele and Sartaj, 2018; Capson-Tojo et al., 2018). Based on that (Akindele and Sartaj, 2018) have proposed that the initial archaeal composition of the inoculum plays an important role during the reactor start-up in order to achieve a stable AD process at high ammonia levels. Hence, we can infer that ammonia is the major but

not the only driving force to shape the methanogenic communities of the AD processes, the other factors determining the methanogens dominance in digesters need further investigation

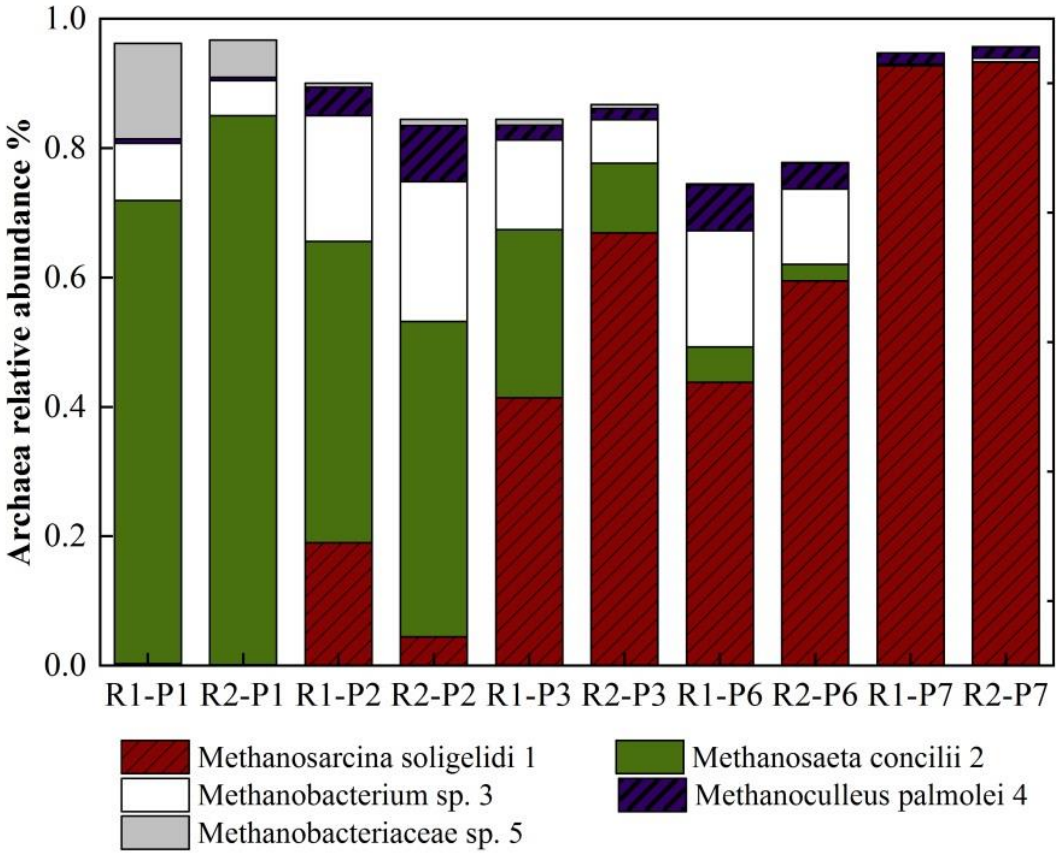

Figure 4. Archaeal relative abundance (%) in different phases of reactor R1and R2 at species level.

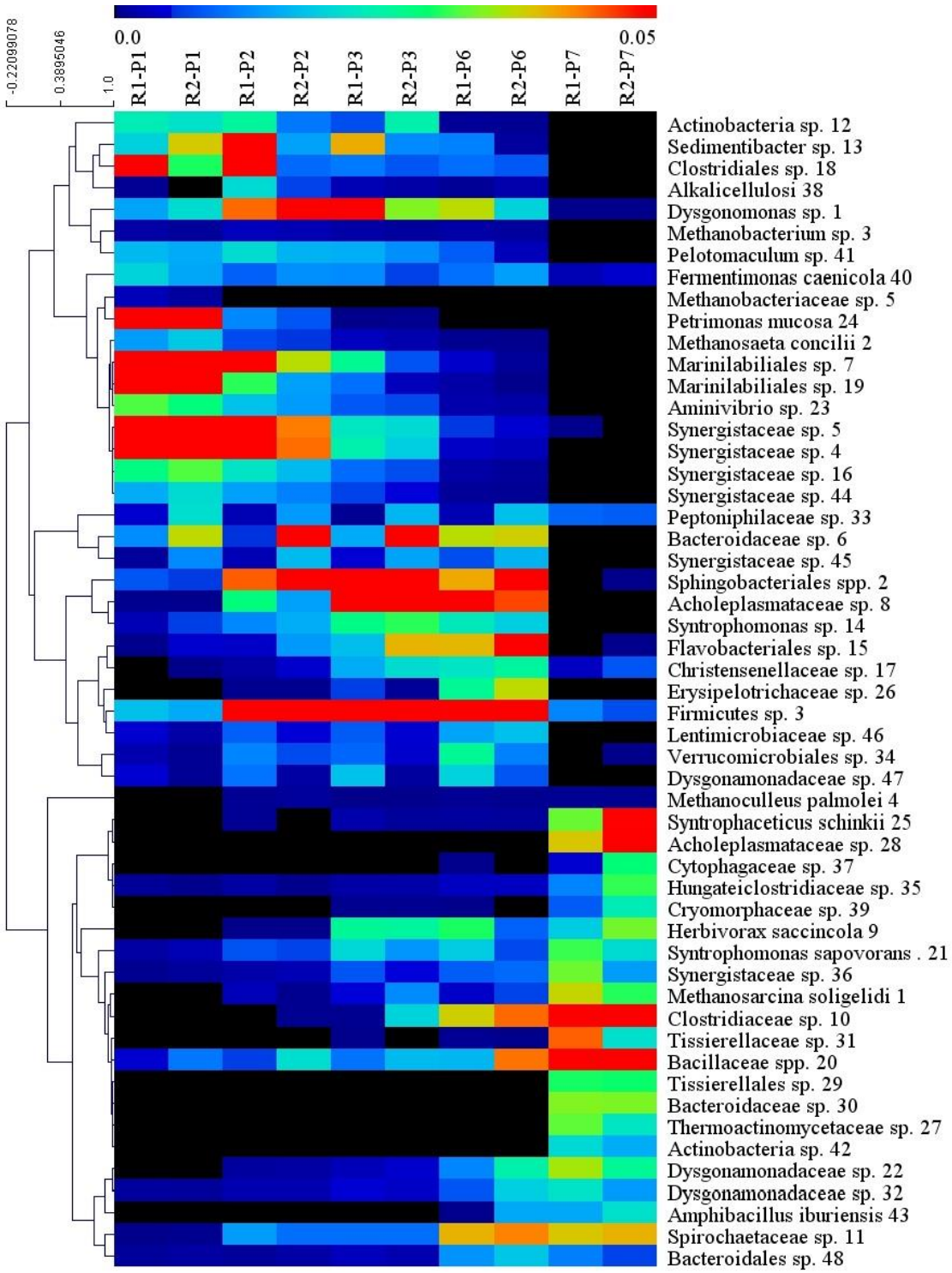

Figure 5. Hierarchical cluster analysis of interesting bacteria and archaea in R1and R2 at the different ammonia level.

3.2.2. Trends in bacterial populations

From P1 to P7, the addition of ammonia resulted in a dramatic change of the bacterial community (Fig. 5). To be more specific, at P1, the predominant phylum was *Bacteroidetes* with around 41.80% of relative abundance, followed by the *Synergistetes* (24.78%) and *Firmicutes* (21.99%). As the ammonia levels increased, *Firmicutes* became the most dominant phylum with a relative abundance of 69.12% at P7. *Firmicutes* spp., which mediates the acidogenesis step of the AD process, have a recorded robustness to high TAN concentrations (Frank et al., 2016; Chen et al., 2018). On contrary, *Bacteroidetes* spp. and *Synergistetes* spp. abundances declined to 15.20% and <1%, respectively, at P7; which indicated that both *Synergistetes* and *Bacteroidetes* phyla members were vulnerable to the elevated ammonia levels despite the stepwise acclimatization process.

At species-level, the relative abundance of *Bacillaceae* sp. 20 and *Syntrophaceticus schinkii* 25 showed a dramatic increase from undetectable levels (prior to P5) to 12% (P7). *Bacillaceae* sp. 20 was found to be similar to novel uncultured phylotype of syntrophic acetate oxidizing bacteria (SAOB) with 100% identity (Westerholm et al., 2010); while, *Syntrophaceticus schinkii* 25 was proposed to be syntrophic partner of a hydrogenotrophic methanogen (e.g. *Methanoculleus* sp.4), to perform methanogenesis by interspecies hydrogen transfer (Wagner et al., 2013). Thus, the extreme low abundance of strictly hydrogen-utilizing methanogens did not match the increased abundance of these two acetate oxidizing bacteria (SAOB). At the same time, *M. soligelidi* was found to have a faster growth rate on H_2/CO_2 compared to acetate (Örlygsson et al., 1996). Thus, it seems that *M. soligelidi* utilised H_2/CO_2 to perform methanogenesis in synergy with *Bacillaceae* sp. 20 and *Syntrophaceticus schinkii* 25 (SAOBs). Specifically, the low

abundance of these two SAOBs at P1 to P5 was due to the lack of H_2 consumers between P1-P5; therefore, when *M. soligelidi* shifted to a hydrogenotrophic metabolism, the growth of these two SAOBs was stimulated.

Consequently, other acidogenic bacteria, *Clostridiaceae* sp. 10 (92% similarity with *Clostridium acetireducens*), which exclusively uses acetate during degradation of the branched-chain amino acids and alanine to produce butyrate and H_2 (Vartoukian et al., 2007), became the most dominant OTU with relative abundance of 15.5% at P7. The dominance of this species can be attributed to the unique composition of the reactors, containing both VFA and protein-rich substrates. In addition, two other OTUs with higher ammonia tolerance, i.e. *Tissierellales* sp. 30 (3%) and *Bacteroidaceae* sp. 31 (3.4%), were observed with high relative abundance at P7. *Tissierellales* sp. 30 and *Bacteroidaceae* sp. 31 (90% and 92% identity to *Sporanaerobacter acetigenes* and *Bacteroides propionicifaciens*, respectively (Wojcieszak et al., 2017)), were reported to be important acetate and H_2-producing bacteria (Xia et al., 2014; Wojcieszak et al., 2017). The dominancy of these H_2-producing bacteria (e.g. *Clostridiaceae* sp. 10, *Bacillaceae* sp. 20 and *Syntrophaceticus schinkii* 25, *Tissierellales* sp. 30, *Bacteroidaceae* sp. 31) was the result of the potential hydrogenotrophic pathway shift of *M. soligelidi*.

Overall, this microbial community redundancy and versatile metabolic pathway were the major reasons to maintain efficient and stable methane production, without loss of functionality or metabolic flexibility during ammonia acclimatization from 1.1 to 7 g NH^+_4-N/L. Clearly, 8.5 g NH^+_4-N/L was a threshold that introduced a suboptimal AD process and subsequently lead to the sharp drop of the microbial diversity at 9.5 g NH^+_4-N/L.

4. Conclusions

This study demonstrated a successful stepwise acclimatization up to 8.5 g NH_4^+-N/L during AD of the OFMSW. Microbiological analyses showed that ammonia load was identified as the main factor to shape microbial composition. The influence were characterized by a shift from hydrogenotrophic methanogens (e.g. *Methanobacterium* sp.5) and acetoclastic methanogens (*M. concilii*, only low-acetate affinity microorganisms) that prevailed in the initial inoculum samples at P1, to the dominance of metabolically more versatile and ammonia more tolerant acetoclastic methanogens (*M. soligelidi*) at extreme ammonia levels. The archaeal shift was essential to keep high biomethanation efficiency.

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Figure Legends

Figure 1. a) CH_4 production, and b) VFA variation throughout the experimental period.

Figure 2. a) FAN, b) pH throughout the experimental period.

Figure 3. Alpha Diversity a) based on Chao 1 bias-corrected index; b) Beta diversity of the triplicate samples in R1 and R2. Principal components (PC) 1 and 2 explained 53% and 30% of community variation, respectively.

Figure 4. Archaeal relative abundance (%) in different phases of reactor R1and R2 at species level.

Figure 5. Hierarchical cluster analysis of interesting bacteria and archaea in R1and R2 at the different ammonia level.