

The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation

Maspolim, Yogananda; Zhou, Yan; Guo, Chenghong; Xiao, Keke; Ng, Wun Jern

2015

Maspolim, Y., Zhou, Y., Guo, C., Xiao, K., & Ng, W. J. (2015). The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation. *Bioresource technology*, 190, 289-298.

<https://hdl.handle.net/10356/106226>

<https://doi.org/10.1016/j.biortech.2015.04.087>

© 2015 Elsevier. This is the author created version of a work that has been peer reviewed and accepted for publication by *Bioresource Technology*, Elsevier. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [<http://dx.doi.org/10.1016/j.biortech.2015.04.087>].

Downloaded on 23 Jan 2021 13:47:32 SGT

Accepted Manuscript

The Effect of pH on Solubilization of Organic Matter and Microbial Community Structures in Sludge Fermentation

Yogananda Maspolim, Yan Zhou, Chenghong Guo, Keke Xiao, Wun Jern Ng

PII: S0960-8524(15)00613-6
DOI: <http://dx.doi.org/10.1016/j.biortech.2015.04.087>
Reference: BITE 14928

To appear in: *Bioresource Technology*

Received Date: 25 February 2015
Revised Date: 20 April 2015
Accepted Date: 24 April 2015

Please cite this article as: Maspolim, Y., Zhou, Y., Guo, C., Xiao, K., Ng, W.J., The Effect of pH on Solubilization of Organic Matter and Microbial Community Structures in Sludge Fermentation, *Bioresource Technology* (2015), doi: <http://dx.doi.org/10.1016/j.biortech.2015.04.087>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**The Effect of pH on Solubilization of Organic Matter and Microbial Community Structures
in Sludge Fermentation**

Yogananda Maspolim^{a, b}, Yan Zhou^{a, b}, Chenghong Guo^{a, b*}, Keke Xiao^{a, b}, Wun Jern Ng^{a, b**}

^a Advanced Environmental Biotechnology Centre, Nanyang Environment and Water
Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141

^b School of Civil and Environmental Engineering, Nanyang Technological University, 50
Nanyang Avenue, Singapore 639798

* Corresponding Author: Guo Chenghong (CHGUO@ntu.edu.sg)

**Corresponding Author: Ng Wun Jern (WJNg@ntu.edu.sg)

Corresponding Authors Tel.: +65 65921837 (Guo Chenghong)

+65 67906813 (Ng Wun Jern)

Abstract

Sludge fermentation between pH 4 and 11 was investigated to generate volatile fatty acids (VFA). Despite the highest sludge solubilization of 25.9% at pH 11, VFA accumulation was optimized at pH 8 (12.5% out of 13.1% sludge solubilization). 454 pyrosequencing identified wide diversity of acidogens in bioreactors operated at the various pHs, with *Tissierella*, *Petrimonas*, *Proteiniphilum*, *Levilinea*, *Proteiniborus* and *Sedimentibacter* enriched and contributing to the enhanced fermentation at pH 8. Hydrolytic enzymatic assays determined abiotic effect to be the leading cause for improved solubilization under high alkaline condition but the environmental stress at pH 9 and above might lead to disrupt biological activities and eventually VFA production. Furthermore, molecular weight (MW) characterization of the soluble fractions found large MW aromatic substances at pH 9 and above, that is normally associated with poor biodegradability, making them disadvantageous for subsequent bioprocesses. The findings provided information to better understand and control sludge fermentation.

Highlights

- pH 11 gave highest solubilization but the optimal VFA accumulation was at pH 8.
- Low biological activity and high MW substance production dissuaded use of pH > 8.
- Different acidogens were enriched according to the operating pH.
- Enrichment of specific genera at pH 8 maximized VFA accumulation.

Keywords

Sludge fermentation; pH; volatile fatty acids (VFA); size exclusion chromatography (SEC);
454 pyrosequencing

Abbreviations

AD anaerobic digestion

BNR biological nutrient removal

COD chemical oxygen demand

DGGE denaturing gradient gel electrophoresis

DNA deoxyribonucleic acids

EPS extracellular polymeric substances

FISH fluorescence in situ hybridization

MW molecular weight

qPCR quantitative polymerase chain reaction

rRNA ribosomal ribonucleic acids

RI	refractive index
SEC	size exclusion chromatography
SRT	solids retention time
TS	total solids
TSS	total suspended solids
UV	ultraviolet
VFA	volatile fatty acids
VS	volatiles solids
VSS	volatile suspended solids
WAS	waste activated sludge

ACCEPTED MANUSCRIPT

1. Introduction

Sludge treatment and its disposal remain one of the biggest challenges in the wastewater treatment industry, and could account for up to 50% of a wastewater treatment plant's operating cost (Appels et al., 2008). Municipal sludge is often a mixture of primary and secondary (waste activated sludge, WAS) sludges at many wastewater treatment plants. Microbial fermentation could be utilized to reduce the organic content, produce volatile fatty acids (VFA) and subsequently methane. The VFA can be used as carbon source for biological nutrient removal (BNR) processes or biopolymer production (Jiang et al., 2009; Li et al., 2011), while methane is a source of renewable energy (Appels et al., 2008).

Recently, extensive studies had assessed the impact of pH to improve waste activated sludge (WAS) fermentation in batch configuration between pH 4 and 12 at 21°C (Chen et al., 2007), 28°C (Jie et al., 2014), 35°C and 55°C (Zhang et al., 2009; Zhang et al., 2010).

According to these studies, alkaline condition was consistently reported to be more efficient than acidic condition for sludge solubilization, and the highest solubilization was found at the highest pH value tested. VFA accumulation was found to reach maximum at pH 10 at 21°C and 28°C after 8 to 10 days (Chen et al., 2007; Jie et al., 2014). At higher temperature, Zhang et al. (2009) reported that sludge fermentation at 35°C yielded the highest VFA accumulation at pH 9 after 5 days and even higher VFA concentrations at 55°C at pH 8 after 9 days.

Furthermore, Zhang et al (2009) also found that the VFA accumulation did not increase despite of higher solubilization observed at pH 10 and above at mesophilic (35°C) temperature under batch configuration treating WAS. Yu et al. (2008a) found the abiotic effect was the leading cause of the enhanced sludge solubilization at pH 10. Too high pH

value could actually impair the biological microbial fermentation activities as acidogens generally functioned from pH 4 to 8.5 (Appels et al., 2008). However, Yuan et al (2006) established that some degree of biological activities was still retained at pH 10. It is thus necessary to understand the extent of biotic activities during sludge fermentation in milder acidic and alkaline conditions (between pH 4 and 10). This would improve the understanding of biotic activities contribution to sludge solubilization and VFA production, which may potentially reduce chemical usage during sludge fermentation. Besides microbial activity, pH 2.5, 9.8 and 11.8 had been found to influence the molecular weight (MW) distributions of solubilized organic matters released from the sludge, which consequently affected its biodegradability (Eskicioglu et al., 2006; Xie et al., 2014). Similarly, the MW distributions of the soluble fractions between pH 4 and 10 have not been evaluated. Understanding the sludge solubilization products and its solubilization mechanism at different pHs could help to better control and optimize the sludge fermentation process.

In addition, production of VFA is driven by the concerted effort of various acidogenic microorganisms. Investigation of the microbial community structures at different pHs during WAS fermentation have been reported previously. The studies had been performed with batch configuration between pH 4 and 12 at 28°C using denaturing gradient gel electrophoresis (Jie et al., 2014) and semi-continuous configuration at pH 8 (55°C), 9 (35°C) and 10 (21°C) using clone library and fluorescence in situ hybridization (FISH) (Zhang et al., 2010). However, the results could not yet establish a proper link between the roles of microbial populations at different pHs to the performance of sludge fermentation.

Correlation of the identified microorganisms with their possible functions during sludge fermentation at different pHs would provide valuable insights into the underlying biological

reactions. This study used 454 pyrosequencing to gather more sequencing reads than would have been possible with DGGE or other cloning related methods. This way, a more comprehensive representation of the microbial community could be achieved.

The feed for this study would be the mixture of primary and secondary sludges, commonly found at many wastewater treatment plants. This study aims to evaluate the characteristics of the soluble organic matter during semi-continuous mixed sludge fermentation process under mesophilic (35°C) condition and 3 days solids retention time (SRT) at different pHs. Particular attention is given on the organic compositions and the MW distributions. Mesophilic condition is operated in this study so that the process could be readily applied to existing mesophilic anaerobic digesters in the tropical climate. Batch experiments had also demonstrated that the bulk of sludge acidogenesis of waste activated sludge at 35°C was achieved within 3 to 4 days (Zhang et al., 2009; Zhang et al., 2010). Hence, SRT of 3 days is chosen for the sludge fermentation in this study. The microbial community acclimated at different pHs will also be characterized to give microbiological insight to the sludge fermentation process.

2. Materials and Methods

2.1. Bioreactor operation

Semi-continuous bioreactors were operated in 1 L glass bottles with 0.9 L working volume for 106 days. 0.3 L of sludge was withdrawn from each reactor as they were mixed and replaced with 0.3 L of feed sludge daily to make SRT of 3 days. Bioreactors were incubated on a temperature controlled shaker at 35°C and 120 rpm. One bioreactor was operated without pH adjustment and served as control, while eight bioreactors were controlled at pH

4, 5, 6, 7, 8, 9, 10 and 11 using 5 M hydrochloric acid or 5 M sodium hydroxide. The pH adjustments were done throughout the experiment, twice every day with 12 hour intervals. However, a fixed pH value could not be maintained. The pH variations and chemical dosage are presented in **Table 1**. The bioreactors were initially seeded with anaerobic sludge treating sewage sludge from a local full-scale wastewater treatment plant and purged with nitrogen to create the anaerobic condition. The feed sludge is sewage sludge, composed of mixed primary and secondary sludge from the same plant. The feed sludge characteristics were 25.4 ± 3.0 g TS L⁻¹; 20.3 ± 2.5 g VS L⁻¹; $37,200 \pm 2,800$ mg tCOD L⁻¹; $2,000 \pm 400$ mg sCOD L⁻¹; and pH 5.8 to 6.0.

(*insert **Table 1** here)

2.2. Sludge fractioning protocol

Sludge fractioning protocol was performed to extract the extracellular polymeric substances (EPS), namely the pellet, tightly-bound EPS, loosely-bound EPS and slime fractions as described by Yu et al. (2008b) for enzymatic activity measurements. The tightly-bound EPS, loosely-bound EPS and slime fractions were then filtered through 0.45 µm nylon filters to remove particulates. All EPS fractions were resuspended in phosphate buffered saline solutions, pH adjusted according to the pH of their respective bioreactors. All EPS fractions were subsequently stored at 4°C before further use.

2.3. Analytical methods

Chemical oxygen demand (COD), totals solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS) were measured in accordance with the standard methods (APHA et al., 2005). Soluble fraction of sludge sample to measure soluble COD

(sCOD), soluble carbohydrates and soluble proteins were prepared by centrifugation at 12,000 xg for 5 min and the supernatant was filtered through 0.45 µm nylon filter. Soluble protein and carbohydrate concentrations were measured using Lowry-Folin and phenol-sulphuric acid methods, respectively (Feng et al., 2009). The COD conversion factors for carbohydrate (glucose) was 1.07 mg COD mg glucose⁻¹ and protein (bovine serum albumin, BSA) was 1.5 mg COD mg BSA⁻¹ (Feng et al., 2009). VFA concentrations and biogas compositions were measured as described previously using gas chromatography (GC) (Agilent, USA) after filtering the soluble fraction through 0.2 µm nylon filter (Maspolim et al., 2014). Daily biogas volume produced was collected in tedlar gas bags and measured with a wet gas meter (Ritter, Germany). The average values were calculated after the bioreactors had achieved steady-state from the 37th day. The extent of solubilization was calculated as described by Ge et al. (2011), which is expressed as

$$\text{Extent of solubilization \%} = \frac{\text{COD}_{\text{CH}_4} + \text{COD}_{\text{So}} - \text{COD}_{\text{Si}}}{\text{COD}_{\text{Ti}} - \text{COD}_{\text{Si}}} \times 100\% \quad (1)$$

where COD_{CH₄} = methane produced a day, as mg COD based on 0.38225 L CH₄ g COD⁻¹ at 25°C (temperature during biogas volume measurement); COD_{Si} = soluble COD concentration of inlet; COD_{So} = soluble COD concentration of outlet; COD_{Ti} = total COD concentration of inlet.

Amylase and protease activities were measured to represent enzymatic activities produced by microorganisms to hydrolyse carbohydrate and protein, respectively (Yu et al., 2008a).

These enzymatic tests were performed to all the sludge fractions. Amylase activity was analysed with enzymatic assay of α-amylase (Sigma-Aldrich, USA) using 3, 5-dinitrosalicylic acid colorimetric method with soluble starch (S2630, Sigma-Aldrich, USA) as substrate and

30 minutes incubation. Protease activity was analysed with universal protease activity assay (Sigma-Aldrich, USA) using Folin's & Ciocalteu's phenol colorimetric method with casein (C7078, Sigma-Aldrich, USA) as substrate and 60 minutes incubation.

Size exclusion chromatography (SEC) was performed using high-performance liquid chromatography (HPLC) (Agilent, USA) following methods described by Tian et al. (2014). Refractive index (RI) and ultraviolet (UV) light at 254 nm were used as detectors. The use of RI detector allowed universal visualization of materials as long as sufficient concentrations were present. UV detector at 254 nm allowed for specific visualizations of aromatic compounds such as proteins and humic acids (Yang et al., 2013). The molecular weight (MW) was calibrated using polyethylene glycol and polyethylene oxide standards with MW of 500, 70, 4, 0.6 and 0.1 kDa. A linear relationship could be derived (Tian et al., 2014) and was expressed as

$$\text{Log (molecular weight)} = 9.8223 - 0.6748 \times (\text{retention time}) \quad (2)$$

2.4. DNA extraction and quantitative PCR (qPCR)

Genomic DNA was extracted from the seed sludge, feed sludge and bioreactors after 47 and 77 days of bioreactor operation. PowerSoil DNA isolation kit was used to extract the DNA in duplicates, following the manufacturer's instructions (MoBio, Singapore). Extracted DNA was then stored at -20°C before further analysis. Quantitative PCR (qPCR) was performed using primers and TaqMan probes targeting the 16S ribosomal ribonucleic acids (rRNA) gene of universal *Bacteria* and universal *Archaea*, as described previously (Maspolim et al., 2014). qPCR was used to evaluate the impact of pH on archaeal and bacterial population quantities.

2.5. 454 pyrosequencing and the bioinformatics analysis

The extracted genomic DNA was sent to Research Testing Laboratory (RTL, USA) for bacterial tag-encoded FLX amplicon pyrosequencing as described by Dowd et al. (2008). 16S rRNA gene universal bacterial primer set (341F and 907R, targeting V3-V5 hypervariable region) (Muyzer et al., 1993) and 16S rRNA gene universal archaeal primer set (517F and 909R, targeting V4-V5 hypervariable region) (Baker et al., 2003) were used for the PCR amplification. The sequences were archived in NCBI Sequence Read Archive (SRA) with the accession SRP055486. The program Mothur v 1.33.3 was used to prepare the raw data for analysis in accordance with Mothur 454 SOP (Schloss et al., 2011). Raw sequences obtained from RTL were initially trimmed to exclude sequences with at least 1 ambiguous base calls, more than 1 barcode mismatch, 2 primer mismatch, 8 homopolymeric bases and if they contained average quality score below 25 over a 50 bp sliding window. Sequences were then aligned using the respective SILVA bacterial and archaeal database, and trimmed again so that all sequences started from the same aligned position with at least half of the expected read length. The “pre-cluster” function was subsequently used to merge sequences with 1 bp difference. Chimeras were removed UCHIME using database-independent approach. OTUs were clustered at 97% sequence similarity with average neighbouring clustering algorithm. Normalization of sample size was conducted by “sub.sample” function in Mothur by resampling the same number of reads for each sample, based on the smallest sample size. Taxonomic classification at the phylum and genus level were done with naïve Bayesian classifier method using RDP training set 9 alignment database and taxonomy, bootstrapped 1,000 times with 50% cutoff. Heatmap was also constructed with heatmap.2 function from R using the bacterial data output from Mothur,

whereby the OTUs containing less than 30 reads were removed for visualization purpose (Fig. SA). The OTUs with their genus identification were aligned on the y-axis and were hierarchically clustered using Bray-Curtis algorithm. The heatmap was used to identify important classified bacterial genus during the microbial community analysis.

3. Results and discussion

3.1. Effect of pH on the solubilization of organic matter

(*insert Fig. 1. here)

Higher mixed sludge solubilization was achieved with higher pH, whereby pH 11 (the highest pH tested in this study) achieved the highest solubilization at 25.9%. The highest solubilization in acidic condition (pH 5) was at 8.2% while the neutral bioreactor achieved 6.8% solubilization (Fig. 1A). Consistent with previous findings treating WAS, alkaline condition was better for sludge solubilization than acidic and neutral conditions between pH 4 and 11 (Chen et al., 2007; Jie et al., 2014). The organic compositions of the sludge soluble fractions tested in this study were methane, VFA, carbohydrates and proteins. Other components not accounted for were categorised as others. Zhang et al. (2009) found elevated concentrations of ethanol and lactic acids during WAS fermentation process in both increasing acidic and alkaline conditions, which may have contributed to this “other” component. VFA formed almost the entire soluble organic composition in the control and pH 5 to 7 (up to 7.4%), indicating effective acidogenesis reactions (Fig 1A). The VFA component stabilized at around 12% from pH 8 to 10 even though there was increasing sludge solubilization, as proteins, carbohydrates and other components gradually increased (Fig 1A). This showed that higher solubilization caused by the alkali action did not

necessarily translate to higher VFA accumulation at pH above 8. Indeed, lower VFA accumulation was observed at pH 11, where VFA formed 8.9% solubilization while carbohydrates, proteins and other components formed 2.1%, 6.5% and 8.3%, respectively (**Fig 1A**). Poor acidogenesis was also observed at pH 4, where VFA formed only 1.9% out of 5.2% of sludge solubilization.

Acetic and propionic acids were always the predominant accumulated VFA species under all conditions, except in the control, pH 6 and 7 bioreactors where part of the acetic acid was converted into methane (**Fig. 1B**). Butyric and iso-valeric acids followed as the next most predominant VFA species in the bioreactors. Both acetic and propionic acids would be preferred for subsequent BNR, AD or biopolymer production (Jiang et al., 2009; Li et al., 2011).

Methane production in the control, pH 6 and 7 contributed to 0.5% to 0.6% solubilization. It was also detected in bioreactor at pH 8 but at much lower percentage (0.09% solubilization). On the other hand, negligible biogas production was detected at pH below 6 and above 8. Hence, operating pH restricted methanogenic activity, which utilized VFA to produce methane during sludge fermentation.

VFA yields during the mixed sludge fermentation were calculated to be 410 ± 70 mg COD g VSS⁻¹ at pH 8 and 150 ± 40 mg COD g VSS⁻¹ with no pH control. These values were higher compared to similar batch WAS fermentations under 35°C between pH 4 to 11, where the highest VFA yield were obtained at pH 9 (298 mg COD g VSS⁻¹) and the reactor with no pH control gave 60 mg COD g VSS⁻¹ (Zhang et al., 2009). The type of substrate being treated could influence the sludge fermentation. It had been discussed previously that primary sludge, which formed part of the mixed feed sludge in this study, was more easily degraded

than WAS (Zhang et al., 2009). This possibly explained the higher VFA yield obtained in this study compared to the mesophilic batch fermentation treating WAS alone.

(*insert Fig. 2. here)

In terms of total activity, it was found that amylase and protease activities were reduced below pH 6 and above pH 8 (**Fig. 2**). Total enzymatic activities at pH 5 and below, as well as, pH 10 and above were reduced by more than 50%. In contrast, sludge solubilization was increased with higher pH or lower pH beyond the control (pH 6) (**Fig. 1A**), indicating the role of abiotic effect by acid or alkaline dosage to hydrolysis at different pHs. The results also demonstrated the detrimental impact of alkaline condition to the biotic contribution to hydrolysis. It could be inferred that this impact of high pH to bioprocess might lead to the poor acidification efficiency at pH 9 and above. However, further study is required to confirm the effect of pH on the enzymatic activities for acidogenesis reactions. The development of alkaliphilic microorganisms associated with sludge fermentation would be discussed later.

(*insert Fig. 3. here)

Soluble fractions of the control and pH 6 bioreactors gave the lowest RI signals relative to all other bioreactors (**Fig. 3A and 3B**), which was consistent with the extent of solubilization results based on COD in **Fig. 1A**. Different molecular weight (MW) distributions were observed in the bioreactors at different pH. All samples showed a peak with MW size between 0.1 and 4 kDa (**Fig. 3A and 3B**). Meanwhile, another peak was detected from the pH 9, 10 and 11 soluble fractions which had a size larger than 500 kDa (**Fig. 3B**).

Passing the soluble fractions through a UV detector at 254 nm did not yield obvious signals in all samples for the range between the 0.1 and 4 kDa (**Fig. 3C and 3D**), indicating that the substance detected on RI was not aromatic in nature and might represent VFA, as shown in **Fig. 1A**. A large MW peak (> 500 kDa) was, however, observed at pH 9, 10 and 11 (**Fig. 3D**). This large MW peak could have been derived from release of proteinaceous cell fragments or EPS into the soluble fractions (Eskicioglu et al., 2006), which was mediated by the sodium hydroxide dosed. In alkaline condition, proteins were denatured and EPS were solubilized to release the organic contents tightly bound within the sludge matrix. Damage to the microbial cell in the sludge also occurred as alkali induced solubilization of membrane proteins and saponification of the membrane lipids (Mendonca et al., 1994). Previous investigation assessed the biodegradability of soluble fractions at different MW which were derived from WAS pretreated with microwave and thermal hydrolysis (96°C) (Eskicioglu et al., 2006). That study found that the soluble fractions larger than 300 kDa gave lower biodegradation rate than other soluble fractions containing smaller MW molecules due to structural complexity and lower surface area to volume ratio. The presence of such high MW substances would be disadvantageous for subsequent use of the fermentation liquor in BNR processes, biopolymer production or methanogenesis, which required VFA as the ideal carbon source. Hence, the use of fermentation liquor from mixed sludge fermentation at pH above 8 would not be a preferred option and require further examination. Overall, these findings suggested operation of mixed sludge fermentation is recommended at pH 8 at mesophilic (35°C) condition with 3 days SRT.

3.3. Effect of pH on microbial community structures

3.3.1 Microbial quantitative analysis by qPCR

(*insert **Fig. 4.** here)

The archaeal and bacterial DNA copies of the feed sludge influenced the archaeal and bacterial DNA copies in the bioreactors, as higher archaeal and bacterial DNA copies observed at 77 than 47 days in the feed sludge corresponded to higher abundances in the respective bioreactors. In general, both the archaeal and bacterial DNA copies were reduced compared to the feed sludge as the bioreactors were operated, except for the *Archaea* in the control, at pH 6 to 8 and pH 9 at 47 days, and the *Bacteria* in the control, at pH 6 to 9 at 47 days and pH 7 at 77 days (**Fig. 4**). *Archaea* showed the highest abundance at pH 7, followed by the control, pH 6 and pH 8. On the contrary, the sludges at pH 4, 10 and 11 experienced almost one log of reductions in archaeal DNA copy abundance compared to the feed sludge (**Fig. 4A**). As *Archaea* are typically found to be methanogens in anaerobic reactors, the results indicated that pH around neutral were more favourable for the growth of methanogens than acidic or alkaline conditions, hence the methanogenesis recorded around neutral pHs in this study (**Fig. 1A**). It was also noted that despite the similar methane volume produced at pH 6 and 7, the archaeal abundance was slightly higher at pH 7 than 6. This might be attributed to the different methanogenic communities present in the two conditions, which would be described later. Similarly, there were also quantitative reductions to the feed sludge in the bacterial qPCR results. Almost one log reduction was observed at pH 4, 10 and 11 at 77 days and it was halved at pH 5, 8 and 9 (**Fig. 4B**). Since qPCR targeted universal *Bacteria*, it was unclear if the reduction of *Bacteria* abundances was attributed to the loss of microorganisms derived from the feed sludge, or the active acidogenic microorganisms. Nevertheless, qPCR confirmed the influence of acidic and alkaline conditions to the reduction of both archaeal and bacterial population abundances.

3.3.2 Microbial community analysis by 454 pyrosequencing

454 pyrosequencing targeting *Archaea* generated a total of 210,000 sequencing reads in this study. Sequence processing, clustering into OTUs at 97% similarity and normalization by Mothur reduced the number of reads to 1,544 reads in each sample. Taxonomic classification using RDP database resulted in 99% of the OTUs being classified to genus level and the rest as unclassified (**Fig. 5A**). All the archaeal genera identified were known methanogens. *Methanolinea* and *Methanospirillum* were found to be predominant methanogens in the samples collected from all bioreactors including the feed and seed sludges (**Fig. 5B**). The relative abundance of the hydrogenotrophic *Methanolinea* was between 41% and 93%, except at pH 7 and 8 with 28% to 48% relative abundance. Meanwhile, hydrogenotrophic *Methanospirillum*'s relative abundance was between 5% and 35% (**Fig 5B**). Hydrogenotrophic methanogenesis seemed to be the important pathway for methanogenesis with short SRT during sludge fermentation process and this had been observed in previous anaerobic sludge digestion processes (Kim et al., 2013). In addition, *Methanosarcina* was enriched in the control (naturally buffered to between pH 5.8 and 6.2), pH 6 and 7 bioreactors, but negligible in the other bioreactors. These enrichments were consistently observed in both the 47 and 77 days samples. *Methanosarcina* can grow between pH 5 and 8 (De Vrieze et al., 2012) and this coincided with the pH values where it was enriched. Hence, *Methanosarcina* was likely associated with methanogenesis between pH 6 and 7. In contrast, *Methanocorpusculum* was exclusively enriched at pH 7 and 8 (**Fig. 5B**), as hydrogenotrophic *Methanocorpusculum* can grow between pH 6.5 and 7.5 (Anderson et al., 2009). *Methanocorpusculum* appeared to be cultivated better than *Methanosarcina* at pH 7 and 8, and would also likely to be associated with methanogenesis

under these conditions. Characterization of the methanogenic communities by 454 pyrosequencing and their abundances by qPCR demonstrated that methanogens could be sustained and acclimated at pH 6, 7 and 8 with short SRT (3 days), which could be correlated with the methanogenic activities under these conditions.

(*insert Fig. 5. here)

454 pyrosequencing targeting *Bacteria* generated a total of 300,000 sequencing reads in this study. Sequence processing, clustering to 97% similarity and normalization by Mothur reduced the number of reads to 955 reads in each sample. Microbial ecology in the anaerobic process is still yet to be understood as 29% of the bacterial OTUs could not be classified to genus level (**Fig. 5A**). Similar to the archaeal communities, the bacterial communities found in 47 days were related to those in 77 days.

(*insert Fig. 6 here)

(*insert Table 2. here)

Fig. 6 shows that *Parabacteroides*, *Paludibacter* and *Caldisericum* were detected in the seed sludge, feed sludge, control and bioreactors in acidic, neutral and alkaline conditions. These genera had been commonly discovered in anaerobic digesters previously and were found to be involved in VFA production (Krieg et al., 2010). This implied that the feed sludge had developed acidogens which subsequently influenced the bacterial composition of the bioreactors in this study. However, these genera predominating in the feed sludge were largely replaced by other genera in all bioreactors. This proved that the reductions of bacterial DNA copies observed in qPCR at pH 4, 10 and 11 (**Fig. 4B**) were attributable to the death of microorganisms derived from the feed sludge, while specialized microorganisms

were enriched. The semi-continuous sludge fermentation process with 3 days SRT between the control and pH 4 to 11 at 35°C in this study led to the acclimation of heterogeneous bacterial community structures capable of producing VFA. Importantly, the specific genera enriched in the bioreactors in this study were optimal in their respective operating pH conditions (**Fig. 6** and **Table 2**). Hence, operating pH could be considered as one of the selective pressure for the enrichment of these different genera and that there was a wide diversity of microorganisms with acidogenic capabilities from pH 4 to 11.

Under acidic condition, *Hallella* was detected in the control, pH 4, 5 and 6 bioreactors, and *Phascolarctobacterium* in the control, pH 5 and 6, but not under neutral and alkaline conditions (**Fig. 6**). *Prevotella* and *Clostridium_sensu_stricto* were enriched at pH 5 alone, while *Oscillibacter* only at pH 4. *Oscillibacter valericigenes* is known to be a mesophilic, anaerobic fermentative bacterium which produced valeric acid as the main fermentation product from carbohydrates with optimum pH of 6 to 6.5 (Iino et al., 2007). *Hallella* and *Prevotella* are anaerobic *Bacteria* that ferment carbohydrates and some proteins to succinic and acetic acids, which grew at pH 5.5 to 5.7 and 4.6 to 5, respectively (Krieg et al., 2010). The succinic acid produced could in turn be consumed by *Phascolarctobacterium* to produce propionic acid (Vos et al., 2009). Meanwhile, *Clostridium_sensu_stricto* species were grouped as *Clostridium* cluster 1, based on their 16S rRNA fingerprints but displayed a variety of phenotypic characteristics (Vos et al., 2009). Despite the acclimation of acidogens under acidic condition, the enzymatic activity at pH 4 and 5 (**Fig. 2**) and the sludge solubilization mediated by acid dosage here were lower than in alkaline conditions. Consequently, less solubilized organic matters were available to be acidified (**Fig. 1A**). In addition, *Hallella* and *Oscillibacter* were the genera enriched at pH 4, but their optimal

growth conditions were not at pH 4 (Iino et al., 2007; Krieg et al., 2010). The enriched microbial communities at pH 4 might not be functioning optimally and this could also explain its low acidogenic efficiency, as the VFA component contributed only half of the sludge solubilization (**Fig. 1A**).

Under alkaline condition, **Fig. 6** shows that *Tissierella* could be detected at pH 8, 9 and 10. In addition, *Petrimonas*, *Proteiniphilum*, *Levilinea* and *Proteiniborus* could be found at pH 8 and 9. *Tissierella* grew optimally at mesophilic condition and produced acetic, butyric, isovaleric acids at optimum pH of 8.3 (Vos et al., 2009), which was similar condition to the pH range it was enriched in this study. *Levilinea* is an anaerobic microorganism isolated from an anaerobic reactor, which ferments sugars and amino acids into hydrogen, acetic and lactic acids between pH 6 to 7.2 (Yamada et al., 2006). *Petrimonas*, *Proteiniphilum* and *Proteiniborus* are fermentative *Bacteria*, producing mainly acetic acid, hydrogen and carbon dioxide (Krieg et al., 2010; Vos et al., 2009), with the latter two mainly using proteins as fermentative substrate, with tolerable pH ranges of 6 to 10 (Niu et al., 2008). *Petrimonas* grew optimally at pH 7.2 (Krieg et al., 2010). *Sedimentibacter* was only enriched at pH 8. It was known to ferment proteins through Stickland-type reactions between pH 7 and 8.2 to produce VFA (Vos et al., 2009), and hence, was likely involved in the acidogenesis of proteinaceous matter at pH 8. This was consistent with a previous study which utilized stoichiometric analysis to suggest the Stickland-type pathway as the main pathway for VFA production during WAS fermentation in alkaline condition (Liu et al., 2011). These results demonstrated that *Tissierella*, *Petrimonas*, *Proteiniphilum*, *Levilinea*, *Proteiniborus* and *Sedimentibacter* which were enriched at pH 8 were able to grow in mild alkaline condition. Their biotic contribution, as proven by the hydrolytic enzymatic activities in **Fig. 2**, when

combined with the abiotic solubilization mediated by sodium hydroxide dosage at pH 8, were able to yield comparable VFA accumulation to that at pH 10 in this study. Hence, fermentation at pH 8 is recommended.

At higher pH values, *Guggenheimella* was enriched at pH 10, which utilized mainly proteins to produce VFA (**Fig. 6**) (Vos et al., 2009). The enrichment of *Anaerobranca*, *Alkalibaculum* and *Clostridium_XI* were also observed at pH 10 and 11. Both *Anaerobranca* and *Alkalibaculum* were known to produce VFA. *Anaerobranca* was considered an extremophile, able to survive in alkaliphilic environment (pH 6 to 10.5) and sodium chloride concentration up to 6% (w/v) (**Table 2**) (Vos et al., 2009). Sodium plays a significant role in substrate uptake and ATP generation for alkaliphiles, including *Anaerobranca* (Kevbrin et al., 2005). High sodium in the bioreactors at pH 10 and 11 would have resulted from the high dosage of sodium hydroxide used for pH adjustment and this might drive the enrichment of *Anaerobranca* (Zhang et al., 2010). All the bioreactors operated at pH 9 and above also managed to enrich carbohydrate- and protein-utilizing acidogens, but the additional bioenergetics requirement to survive under the stressed condition of high pH and salinity might lead to the reduced acidogenic activities observed in this study (Kevbrin et al., 2005). The detection of methanogens and acidogenic *Bacteria* in the feed sludge led to the possibility of initiating the sludge fermentation process without inoculum or seed sludge, as had been reported by Morgan-Sagastume et al. (2015). However, the specialized microbial populations enriched at pH 8 were very low or below the detection limit in both the seed inoculum and feed sludge, and hence it could not be clearly determined if these specialized microorganisms originated from the seed or feed sludge. In addition, previous study found that seed inoculum enhanced VFA accumulation compared to without inoculation during

WAS fermentation under mild alkaline condition (pH 9) (Huang et al., 2014), indicating the importance of inoculum for sludge fermentation.

Mixed sludge fermentation at pH 8 provided a balance of abiotic solubilization by sodium hydroxide and preservation of microbial activity to give optimal VFA accumulation in this study, and provided suitable fermentation products for subsequent applications.

Understanding the characteristics of the solubilization product and the underlying microbial communities gave insights for potentially better control and optimization of the sludge fermentation process.

4. Conclusion

VFA accumulation was optimized at pH 8 despite higher solubilization at higher pH, where proteins, carbohydrates and other organic matter formed the remaining organic compositions. The solubilization products after fermentation above pH 8 contained high MW organics with possibly low biodegradability and would be unfavourable for subsequent biological processes. Enzymatic activity results revealed a reduction in biotic hydrolytic activities outside pH 6, 7 and 8 even when suitable acidogens were enriched, indicating the impairment of biological activities. However, *Tissierella*, *Petrimonas*, *Proteiniphilum*, *Levilinea*, *Proteiniborus* and *Sedimentibacter* were enriched at pH 8 and contributed to the highest fermentation efficiency in this study.

Acknowledgement

The authors were grateful to the funding support of National Research Foundation, Singapore for the project “Wastewater Treatment Plants as Urban Eco Power Stations”, and Public Utilities Board (PUB), Singapore for the provision of municipal sewage sludge.

ACCEPTED MANUSCRIPT

References

- Anderson, I.J., Sieprawska-Lupa, M., Goltsman, E., Lapidus, A., Copeland, A., Glavina Del Rio, T., Tice, H., Dalin, E., Barry, K., Pitluck, S., Hauser, L., Land, M., Lucas, S., Richardson, P., Whitman, W.B., Kyrpides, N.C. 2009. Complete genome sequence of *Methanocorpusculum labreanum* type strain Z. *Standards in genomic sciences*, **1**(2), 197-203.
- APHA, AWWA, WPCF. 2005. Standard methods for examination of water and wastewater. New York.
- Appels, L., Baeyens, J., Degrève, J., Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, **34**(6), 755-781.
- Baker, G., Smith, J., Cowan, D.A. 2003. Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods*, **55**(3), 541-555.
- Chen, Y., Jiang, S., Yuan, H., Zhou, Q., Gu, G. 2007. Hydrolysis and acidification of waste activated sludge at different pHs. *Water Research*, **41**(3), 683-689.
- De Vrieze, J., Hennebel, T., Boon, N., Verstraete, W. 2012. *Methanosarcina*: The rediscovered methanogen for heavy duty biomethanation. *Bioresour Technol*, **112**, 1-9.
- Dowd, S.E., Sun, Y., Wolcott, R.D., Domingo, A., Carroll, J.A. 2008. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. *Foodborne Pathogens and Disease*, **5**(4), 459-472.
- Eskicioglu, C., Kennedy, K.J., Droste, R.L. 2006. Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment. *Water Research*, **40**(20), 3725-3736.
- Feng, L., Wang, H., Chen, Y., Wang, Q. 2009. Effect of solids retention time and temperature on waste activated sludge hydrolysis and short-chain fatty acids accumulation under alkaline conditions in continuous-flow reactors. *Bioresource Technology*, **100**(1), 44-49.
- Ge, H., Jensen, P.D., Batstone, D.J. 2011. Temperature phased anaerobic digestion increases apparent hydrolysis rate for waste activated sludge. *Water Research*, **45**(4), 1597-1606.
- Huang, L., Chen, B., Pistolozzi, M., Wu, Z., Wang, J. 2014. Inoculation and alkali coeffect in volatile fatty acids production and microbial community shift in the anaerobic fermentation of waste activated sludge. *Bioresource Technology*, **153**, 87-94.
- Iino, T., Mori, K., Tanaka, K., Suzuki, K.-i., Harayama, S. 2007. *Oscillibacter valericigenes* gen. nov., sp. nov., a valerate-producing anaerobic bacterium isolated from the alimentary canal of a Japanese corbicula clam. *International journal of systematic and evolutionary microbiology*, **57**(8), 1840-1845.
- Jiang, Y., Chen, Y., Zheng, X. 2009. Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by activated sludge submitted to the aerobic feeding and discharge process. *Environmental science & technology*, **43**(20), 7734-7741.
- Jie, W., Peng, Y., Ren, N., Li, B. 2014. Volatile fatty acids (VFAs) accumulation and microbial community structure of excess sludge (ES) at different pHs. *Bioresource Technology*, **152**, 124-129.
- Kevbrin, V., Romanek, C., Wiegel, J. 2005. Alkalithermophiles: a double challenge from extreme environments. in: *Origins*, Springer, pp. 395-412.
- Kim, J., Kim, W., Lee, C. 2013. Absolute dominance of hydrogenotrophic methanogens in full-scale anaerobic sewage sludge digesters. *Journal of Environmental Sciences*, **25**(11), 2272-2280.
- Krieg, N., Ludwig, W., Whitman, W., Hedlund, B., Paster, B., Staley, J. 2010. *Bergey's Manual of Systematic Bacteriology: Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes*, Springer, New York, NY, USA.
- Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., Gu, G. 2011. Pilot-Scale Waste Activated Sludge Alkaline Fermentation, Fermentation Liquid Separation, and Application of Fermentation Liquid To

- Improve Biological Nutrient Removal. *Environmental science & technology*, **45**(5), 1834-1839.
- Liu, H., Wang, J., Liu, X., Fu, B., Chen, J., Yu, H.-Q. 2011. Acidogenic fermentation of proteinaceous sewage sludge: Effect of pH. *Water Research*.
- Maspolim, Y., Zhou, Y., Guo, C., Xiao, K., Ng, W.J. 2014. Comparison of single-stage and two-phase anaerobic sludge digestion systems - performance and microbial community dynamics. *Chemosphere*, in press, doi: 10.1016/j.chemosphere.2014.07.028.
- Mendonca, A.F., Amoroso, T.L., Knabel, S.J. 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. *Applied and environmental microbiology*, **60**(11), 4009-4014.
- Morgan-Sagastume, F., Hjort, M., Cirne, D., Gerardin, F., Lacroix, S., Gaval, G., Karabegovic, L., Alexandersson, T., Johansson, P., Karlsson, A. 2015. Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresource Technology*.
- Muyzer, G., De Waal, E.C., Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and environmental microbiology*, **59**(3), 695-700.
- Niu, L., Song, L., Dong, X. 2008. *Proteiniborus ethanoligenes* gen. nov., sp. nov., an anaerobic protein-utilizing bacterium. *International journal of systematic and evolutionary microbiology*, **58**(1), 12-16.
- Schloss, P.D., Gevers, D., Westcott, S.L. 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PloS One*, **6**(12), e27310.
- Tian, X., Wang, C., Trzcinski, A.P., Lin, L., Ng, W.J. 2014. Interpreting the synergistic effect in combined ultrasonication–ozonation sewage sludge pre-treatment. *Chemosphere*, in press, DOI: 10.1016/j.chemosphere.2014.09.007.
- Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W. 2009. *Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes*, Springer, New York, NY, USA.
- Xie, S., Wu, Y., Wang, W., Wang, J., Luo, Z., Li, S. 2014. Effects of acid/alkaline pretreatment and gamma-ray irradiation on extracellular polymeric substances from sewage sludge. *Radiation Physics and Chemistry*, **97**, 349-353.
- Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., Kamagata, Y. 2006. *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum Chloroflexi. *International journal of systematic and evolutionary microbiology*, **56**(6), 1331-1340.
- Yang, S.-S., Guo, W.-Q., Meng, Z.-H., Zhou, X.-J., Feng, X.-C., Zheng, H.-S., Liu, B., Ren, N.-Q., Cui, Y.-S. 2013. Characterizing the fluorescent products of waste activated sludge in dissolved organic matter following ultrasound assisted ozone pretreatments. *Bioresource Technology*, **131**(0), 560-563.
- Yu, G.-H., He, P.-J., Shao, L.-M., He, P.-P. 2008a. Toward understanding the mechanism of improving the production of volatile fatty acids from activated sludge at pH 10.0. *Water Research*, **42**(18), 4637-4644.
- Yu, G., He, P., Shao, L., Lee, D. 2008b. Extracellular enzymes in sludge flocs collected at 14 full - scale wastewater treatment plants. *Journal of Chemical Technology and Biotechnology*, **83**(12), 1717-1725.
- Yuan, H., Chen, Y., Zhang, H., Jiang, S., Zhou, Q., Gu, G. 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. *Environmental science & technology*, **40**(6), 2025-2029.

- Zhang, P., Chen, Y., Zhou, Q. 2009. Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH. *Water Research*, **43**(15), 3735-3742.
- Zhang, P., Chen, Y., Zhou, Q., Zheng, X., Zhu, X., Zhao, Y. 2010. Understanding short-chain fatty acids accumulation enhanced in waste activated sludge alkaline fermentation: kinetics and microbiology. *Environmental science & technology*, **44**(24), 9343-9348.

ACCEPTED MANUSCRIPT

List of captions

Table 1. The pH variations and chemical dosage of the bioreactors.

Table 2. The pH value and sodium chloride cultivation concentration ranges of the identified genera in the bioreactors by 454 pyrosequencing.

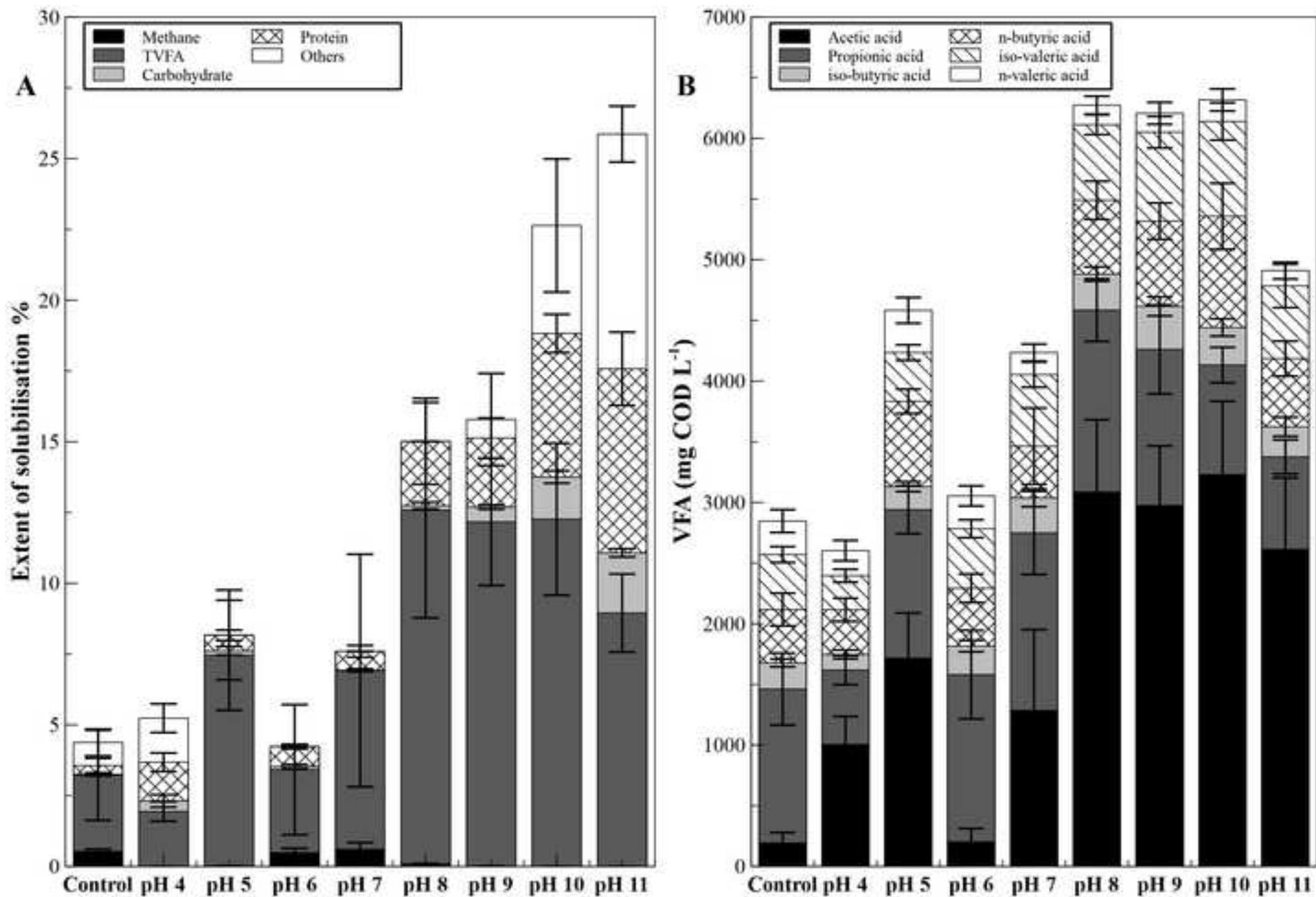
Fig. 1. The average extent of sludge solubilization with its organic composition (a), and the average VFA concentration with its speciation (b) in the bioreactors. Error bars signify the standard deviations for a population sample, $n = 4$.

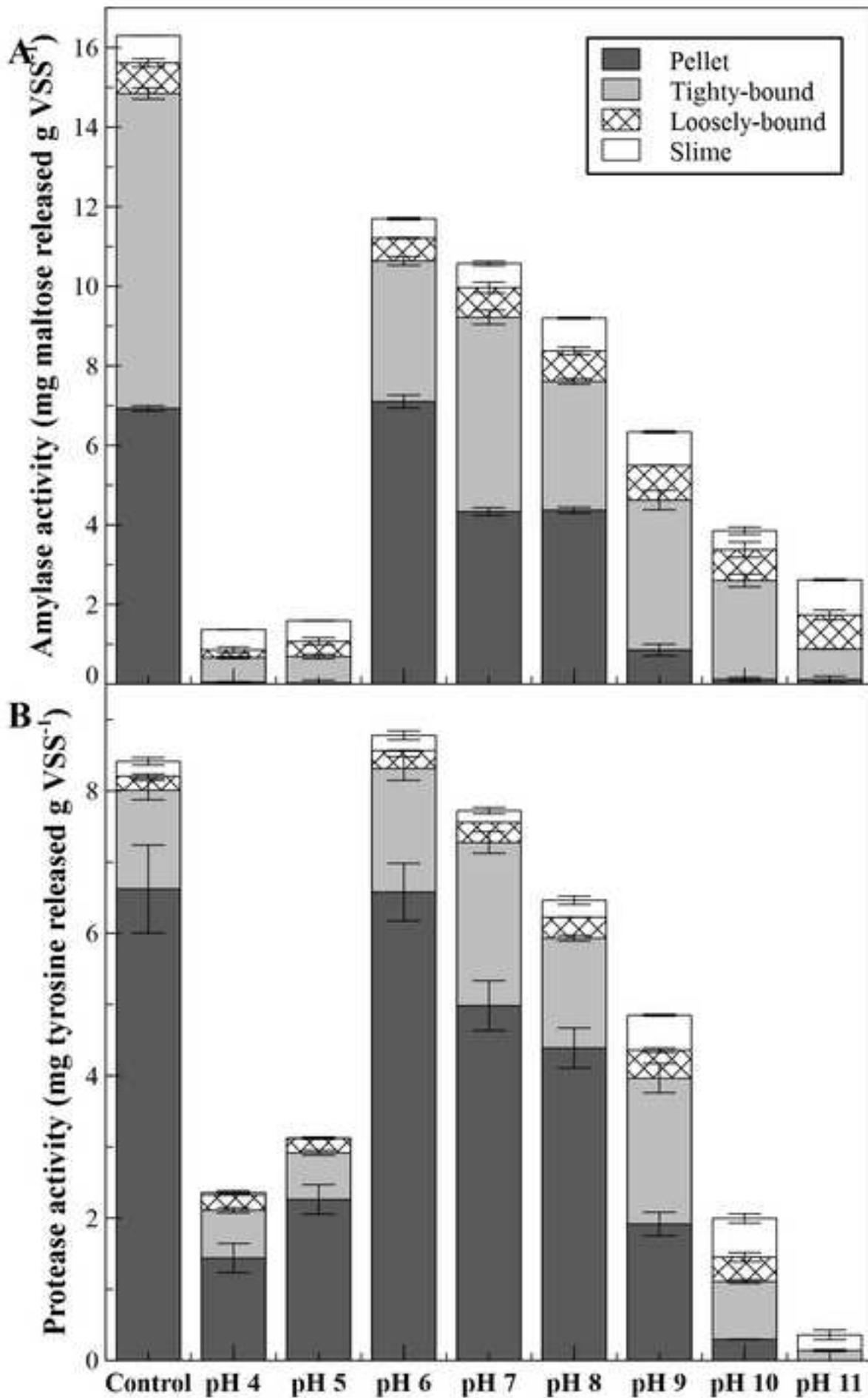
Fig. 2. Amylase (A) and protease activities (B) of EPS fractions from sludges at different pHs. Error bars signify the standard deviations for number of replicates, $n = 3$.

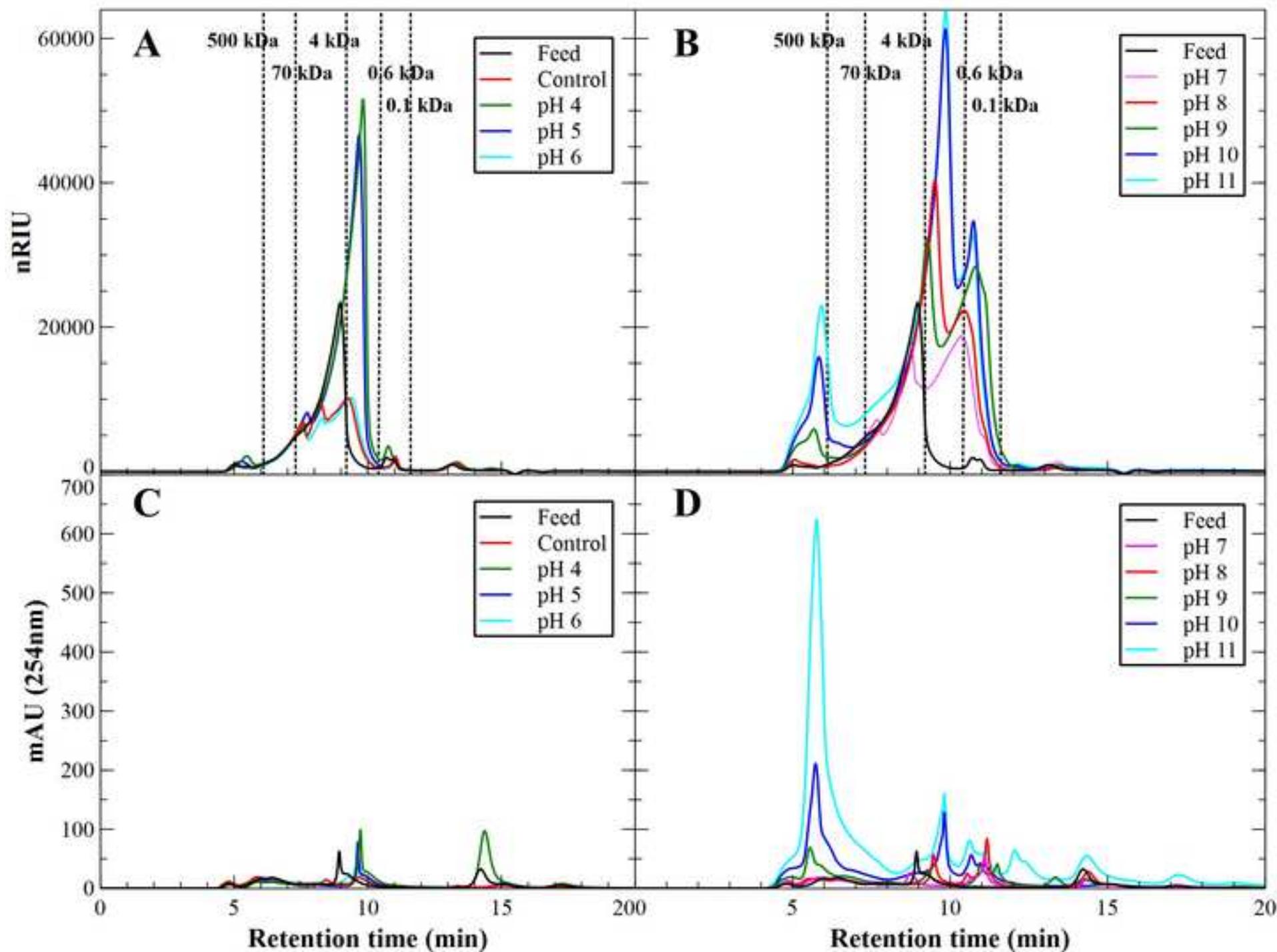
Fig. 3. Size exclusion chromatograms of the soluble fractions from the control and acidic bioreactors with refractive index (a), 254 nm detectors (c); and chromatograms of the soluble fractions from the neutral and alkaline bioreactors at day 77 with refractive index (b), 254 nm detectors (d). The dotted lines represent the retention time of the calibration standards.

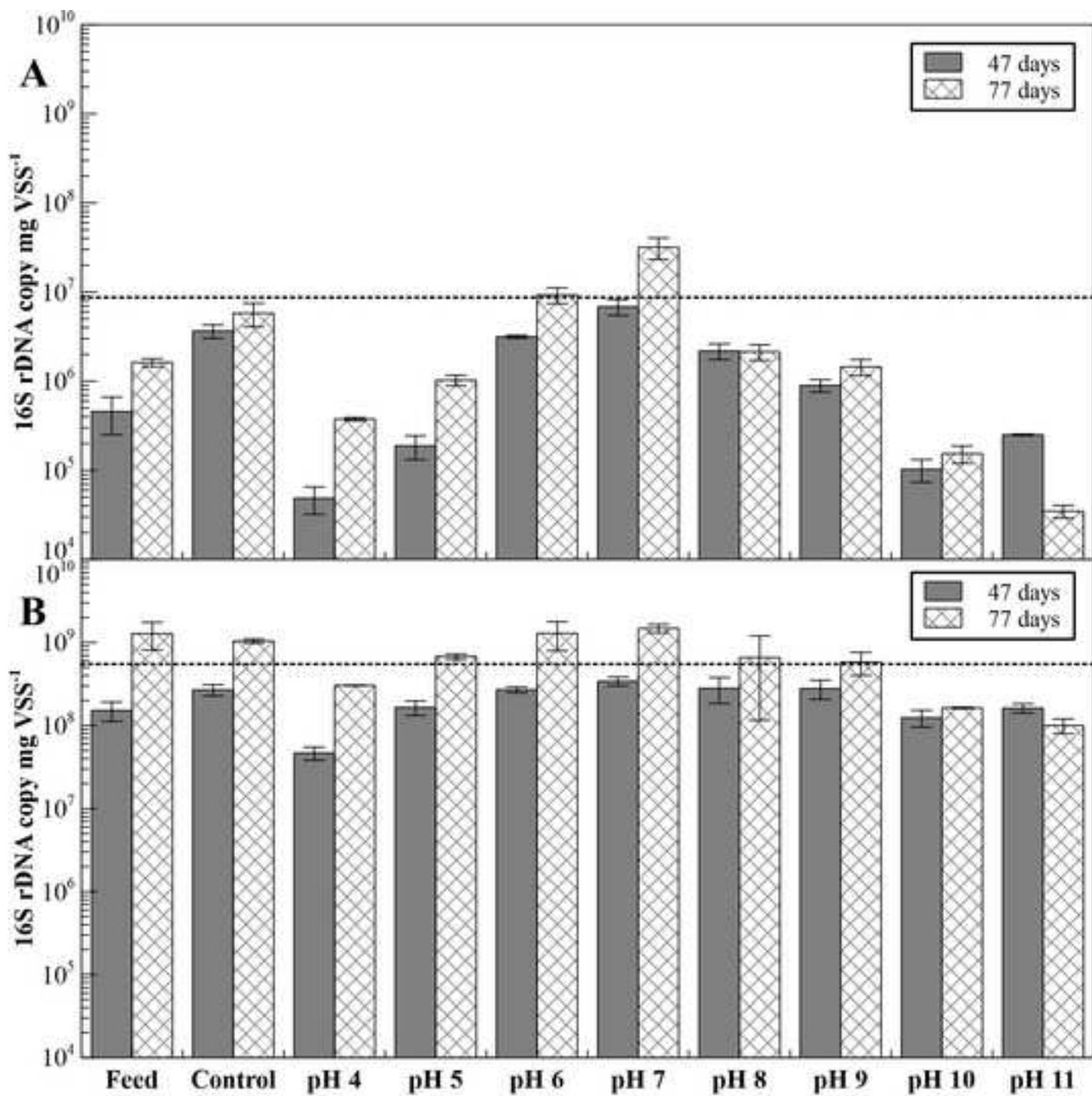
Fig. 4. Microbial quantification by qPCR of the feed sludge and bioreactors at 47 and 77 days, targeting the *Archaea* (A) and *Bacteria* (B). The dotted lines represent the respective archaeal and bacterial quantifications of the anaerobic seed sludge. Error bars signify the standard deviations for number of replicates, $n = 3$.

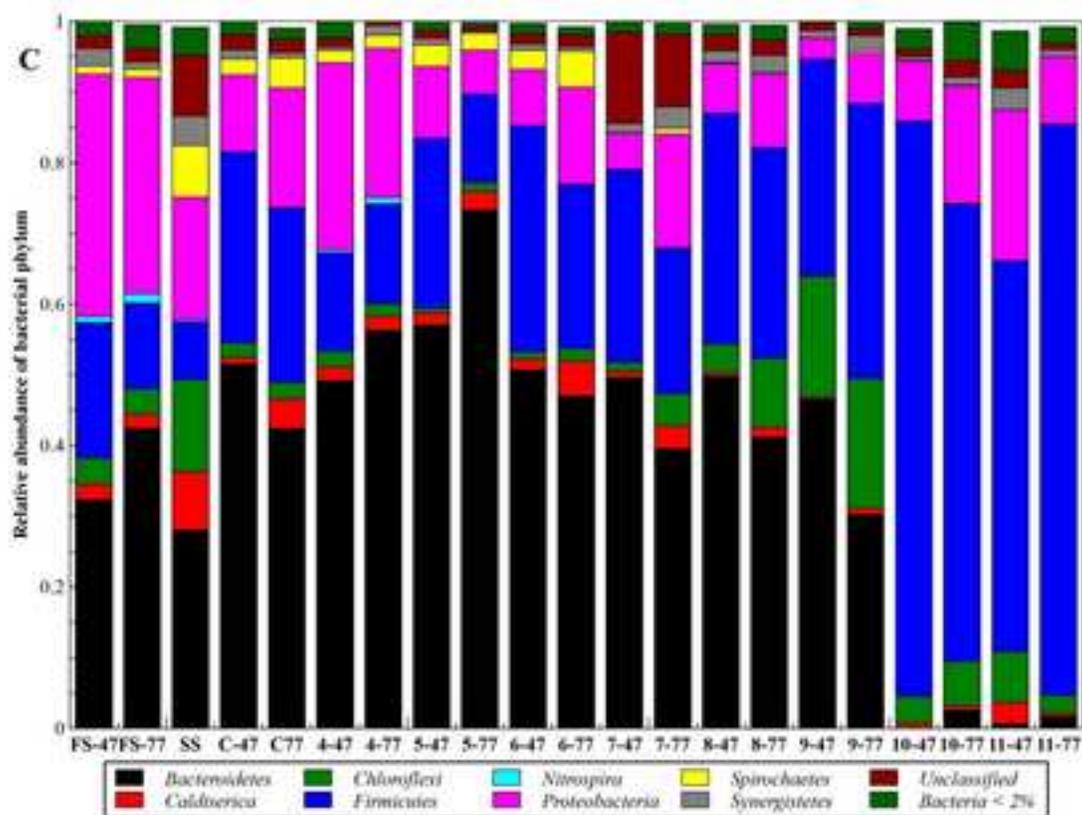
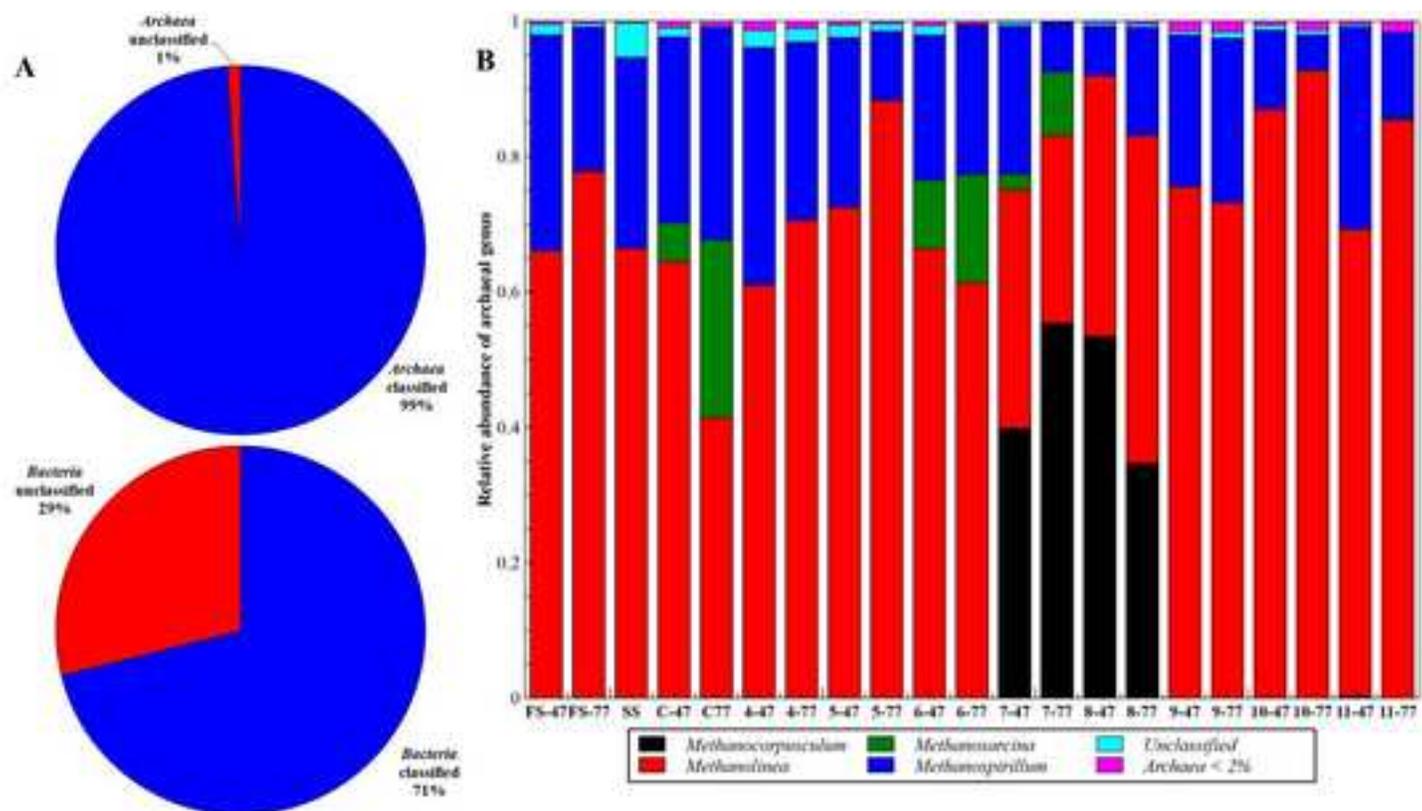
Fig. 5. Distribution of taxonomic classification of *Archaea* and *Bacteria* to genus level (A); the relative abundances of archaeal OTUs with 97% similarity to genus level (B); and the relative abundance of bacterial OTUs with 97% similarity to phylum level (C).











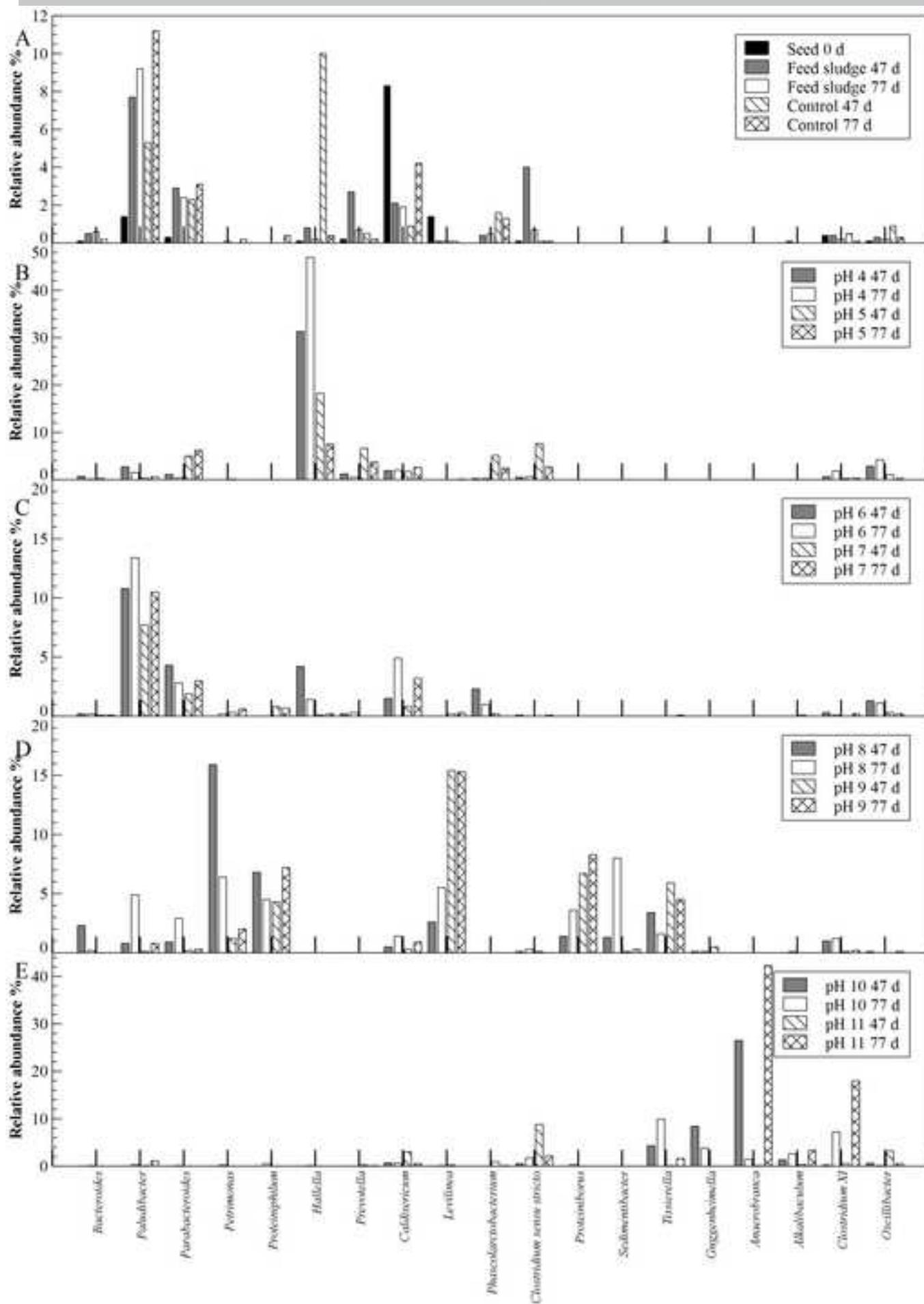


Fig. 6. Relative abundances of bacterial genera in the feed sludge, seed sludge and bioreactors at 47 and 77 days.

ACCEPTED MANUSCRIPT

List of tables**Table 1.** The pH variations and chemical dosage of the bioreactors.

Target pH of bioreactor	pH variations	Chemical dosage
No control	5.8 - 6.3	-
pH 4	3.9 - 4.3	0.05 ± 0.01 g HCl g TS feed ⁻¹
pH 5	4.9 - 5.1	0.01 ± 0.00 g HCl g TS feed ⁻¹
pH 6	5.8 - 6.2	0.01 ± 0.00 g NaOH g TS feed ⁻¹
pH 7	6.5 - 7.2	0.03 ± 0.02 g NaOH g TS feed ⁻¹
pH 8	7.1 - 8.2	0.07 ± 0.02 g NaOH g TS feed ⁻¹
pH 9	8.2 - 9.2	0.1 ± 0.02 g NaOH g TS feed ⁻¹
pH 10	9.2 - 10.2	0.14 ± 0.02 g NaOH g TS feed ⁻¹
pH 11	10.2 - 11.2	0.17 ± 0.02 g NaOH g TS feed ⁻¹

ACCEPTED MANUSCRIPT

Table 2. The pH value and sodium chloride concentration cultivation ranges of the identified genera in the bioreactors by 454 pyrosequencing.

Phylum	Genus	Cultivation pH range	Cultivation NaCl range
<i>Bacteroidetes</i>	<i>Bacteroides</i>	7 to 8.5	N.R.
	<i>Paludibacter</i>	5.0 to 7.6 (optimum 6.6)	0 to 0.5%
	<i>Parabacteroides</i>	5.0 to 8.5 (optimum 7 to 7.5)	0 to 2% 0 to 4%
	<i>Petrimonas</i>	around 7.2 6.0 to 9.7	(optimum 0%)
	<i>Proteiniphilum</i>	(optimum 7.5 to 8)	0 to 5%
	<i>Hallella</i>	5.5 to 5.7	N.R.
<i>Caldiserica</i>	<i>Prevotella</i>	4.6 to 5	N.R.
	<i>Caldisericum</i>	5.5 to 7.5 (optimum 6.5)	0.5 to 1%
<i>Chloroflexi</i>	<i>Levilinea</i>	6 to 7.2	0 to 0.25%
<i>Firmicutes</i>	<i>Phascolarctobacterium</i>	N.R.	N.R.
	<i>Clostridium_sensu_stricto</i>	varied 6.4 to 10 (optimum 8.5 to 8.8)	varied
	<i>Proteiniborus</i>	7 to 8.2	0 to 2%
	<i>Sedimentibacter</i>	6.5 to 8.5 (optimum 8.3)	N.R.
	<i>Tissierella</i>	6.5 to 9 (optimum 7.5 to 8)	N.R.
	<i>Guggenheimella</i>	6 to 10.5 (optimum 8.5 to 9.5)	0 to 6% (optimum 1 to 2.5%)
	<i>Anaerobranca</i>	6.5 to 10.5 (optimum 8 to 8.5)	N.R.
	<i>Alkalibaculum</i>	varied 5.5 to 8.5	varied
	<i>Clostridium_XI</i>	5.5 to 8.5 (optimum 6 to 6.5)	0 to 4%
	<i>Oscillibacter</i>		

Highlights

- pH 11 gave highest solubilization but the optimal VFA accumulation was at pH 8.
- Low biological activity and high MW substance production dissuaded use of pH > 8.
- Different acidogens were enriched according to the operating pH.
- Enrichment of specific genera at pH 8 maximized VFA accumulation.

ACCEPTED MANUSCRIPT