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The role of methanogens in acetic acid production under different salinity conditions

Keke Xiao [a, b], Chenghong Guo [a, b], Yogananda Maspolim [a, b], Yan Zhou [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, Singapore
[b] School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798, Singapore

HIGHLIGHTS

- Salinity in acidogenic reactor was increased to 40‰ to study microbial responses.
- 10^{12} copies mL^{-1} MMB and 4.93×10^{11} copies mL^{-1} MSC were noted in acidogenic reactor.
- The methanogens showed a HAc degradation rate of 3.81 mg COD g^{-1} VSS h^{-1}.
- 20‰ salinity inhibited VFAs production and acetate-utilizing methanogens.

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ABSTRACT

In this study, a fed-batch acidogenic reactor was operated at a 3 d hydraulic retention time (HRT) and fed with alkaline pre-treated sludge to investigate salinity effects on methanogens' abundance, activities and their consumption of produced acetic acid (HAc) and total volatile fatty acids (VFAs). The salinity concentration was increased step-wise by adding sodium chloride. At 3‰ (parts per thousand) salinity, the average produced volatile fatty acids (VFAs) concentration was 2410.16 ± 637.62 mg COD L^{-1} and 2.70 ± 0.36 L methane was produced daily in the acidogenic reactor. Further batch tests indicated methanogens showed a HAc degradation rate of 3.81 mg COD g^{-1} VSS h^{-1} at initial HAc concentration of 1150 mg COD L^{-1}, and showed tolerance up to 16‰ salinity (3.76 g Na^{+} L^{-1}) as indicated by a constant HAc degradation rate. The microbiological study indicated this can be related to the predominance of acetate-utilizing *Methanosarcinaceae* and *Methanomicrobiales* in the reactor. However, with salinity increased to 20‰ and 40‰, increases in VFAs and HAc production and decreases in methane production, methanogens population, acidogenic bacteria population and acidification extent were observed. This study demonstrated presence of acetate-utilizing methanogens in an acidogenic reactor and their high tolerance to salinity, as well as their negative impacts on net VFAs production. The results would suggest the presence of methanogens in the acidogenic reactor should not be ignored and the recovery of methane from the acidogenic reactor needs to be considered to avoid carbon loss.

Abbreviations: Ar, Argon; CH_4, Methane; CO_2, Carbon dioxide; COD, Chemical oxygen demand; COD_{CH4}, Produced amount of CH_4; $COD_{VFA\ eff}$, Amount of COD present as volatile fatty acids in the effluent; $COD_{VFA\ infl}$, Amount of COD present as volatile fatty acids in the influent; d, days; FID, Flame ionization detector; GC, Gas chromatography; h, hours; H_2, Hydrogen; HAc, Acetic acid; HCl, Hydrochloric acid; He, Helium; HF, Hydrogen fluoride; HNO_3, Nitric acid; HRT, Hydraulic retention time; k, Acetic acid degradation rate constants; K_s, Half-saturation constant; MBT, *Methanobacteriales*; min, minutes; MMB, *Methanomicrobiales*; MSC, *Methanosarcinaceae*; MST, *Methanosaetaceae*; N_2, Nitrogen; N.A., Not available; NaCl, Sodium chloride; NaOH, Sodium hydroxide; O_2, Oxygen; qPCR, Quantitative polymerase chain reaction; SCOD, Soluble chemical oxygen demand; s, Seconds; SRT, Solids retention time; TCD, Thermal conductivity detector; $TCOD_{infl}$, Amount of total COD present in the influent; TDS, Total dissolved solids; TS, Total solids; VFAs, Volatile fatty acids; VS, Volatile solids; VS_{infl}, VS concentration in the influent; VS_{eff}, VS concentration in the effluent.

* Corresponding author. Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore, 637141, Singapore.
** Corresponding author. School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798, Singapore.
E-mail addresses: ZhouYan@ntu.edu.sg (Y. Zhou), WJNg@ntu.edu.sg (W.J. Ng).

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1. Introduction

Anaerobic digestion is often a cost-effective method for sludge treatment, and the process comprises hydrolysis, acidogenesis, acetogenesis, and methanogenesis with different microbes acting in concert (Lin et al., 1986). Volatile fatty acids (VFAs) are important intermediates in anaerobic process. These carboxylic acids have many applications in the production of biochemicals, bioplastics and biofuels, and can also be used as energy and carbon sources for biological nutrients removal (Chang et al., 2010). Among all the produced VFAs, acetic acid (HAc) is the main VFAs product (Jiang et al., 2012).

Methanogens, hydrolytic and acidogenic bacteria differ widely in physiology, kinetics, and growth conditions. The optimum pH range for hydrolysis and acidogenesis is 5.5–6.5 (Chyi and Dague, 1994), however, most of methanogens achieve optimum stability and activity at pH of 7–7.5 (Lv et al., 2010). Therefore, there are system configurations which separate the process into two reactors so that kinetic advantages can be achieved for both acid-formers and methanogens (Pohland and Ghosh, 1971). As methanogens are more sensitive to environmental changes and show a slower growth rate compared to hydrolytic and acidogenic bacteria, they are maintained at relatively longer solids retention times (SRTs). With shorter SRTs and acid pH, methanogens could be suppressed and hydrolytic and acidogenic bacteria accumulated so that an acidogenic reactor can be achieved and maintained (Zhang and Noike, 1991). Currently, a lot of researchers have investigated methods to maximize VFAs production in the acidogenic reactor, however, the consumption of produced VFAs by methanogens and the related methane production have been ignored intuitively in the acidogenic reactor (Khiewwijit et al., 2015; Kim et al., 2016; Yin et al., 2016), as conditions therein favor the acidogens, and consumption of produced VFAs by methanogens was regarded as “minor” since methanogens were more vulnerable at shorter HRTs and acid pH thus being potentially suppressed (Zamanzadeh et al., 2013). In fact, complete separation of methanogens from hydrolytic and acidogenic bacteria in the acidogenic reactor is difficult to achieve. The presence of methanogens in the acidogenic reactor (pH 5.5) has been identified (Shimada et al., 2011; Xiao et al., 2013). Moreover, the alkaline or neutral conditions were consistently reported to be more efficient than acid condition for sludge solubilisation (Chen et al., 2007; Zhang et al., 2009, 2010; Jie et al., 2014) in the acidogenic reactor, and the presence of methanogens therein has also been reported (Su et al., 2016). The consumption of produced VFAs in the acidogenic reactor would lead to decrease in net VFAs production (the term net VFAs production is used here to indicate total produced VFAs, namely acidification extent, including the measured produced VFAs concentration and those consumed by methanogens) and acidification efficiency, thus resulting in carbon loss (Maspolim et al., 2015; Yuan et al., 2006). With the presence of methanogens in the acidogenic reactor, there is such a necessity to investigate their dominance, activities and VFAs consumption in the acidification process, thus to better regulate net VFAs production.

Various methods have been applied to improve VFAs production from waste activated sludge, and these methods include controlling pH at alkaline conditions (Chen et al., 2007; Jiang et al., 2007), adding enzymes (Luo et al., 2011), applying ultrasonication (Yan et al., 2010) or ozonation (Yeom et al., 2002) to break up cell walls, adding surfactants (Jiang et al., 2007) and using sodium chloride (NaCl) (Su et al., 2016). Alkaline treatment is advantageous given its simple equipment requirements, high efficiency and convenient operation (Li et al., 2012). It usually acts faster than other methods in increasing sludge solubilisation, and avoids acid-corrosion effects thus allowing for application using mild steel in construction. Alkali manipulation of the sludge matrix is to disrupt particulate organics at high pH, and then causing microbial cells disintegration through chemical means, hydroxyl groups ionization ($-OH \rightarrow -O^-$), and lipids saponification in the cell walls (Neyens et al., 2003). The preferred reagent is sodium hydroxide (NaOH) in most cases. The solubilisation efficiency and related volatile fatty acids production is related to the NaOH dosage (Le et al., 2013). However, disadvantages, such as pH increase and disturbance of the bicarbonate buffer system, could adversely affect the subsequent anaerobic process. Therefore, neutralisation of the alkaline pre-treated sludge with acid is necessary, but this would increase salinity consequently (Li et al., 2012). High salinity had been reported to dis-equilibrate osmotic pressure across cell membranes, causing cell dehydration and plasmolysis, and resulting in deleterious impact on the anaerobic process (Zhang et al., 2012). The acidogenic reactor would receive salinity earlier compared to the following methanogenic reactor. Su et al. (2016) had reported high salinity can enhance the VFAs production of waste activated sludge with more release of soluble protein and polysaccharide. There have, however, been few reports on the impacts of salinity on the acidogenic reactor with focus on the role of methanogens. The effects of salinity on methanogens’ activities and abundance, as well as the related acidification process have not been studied in detail.

The objectives of this study were to investigate (1) the presence of methanogens in an acidogenic reactor and their role in VFAs consumption and (2) the effects of salinity on methanogens’ activities and abundance, as well as the related acidification process.

2. Materials and methods

2.1. Feed stock and reactor operation

The raw feed sludge (pH 5.7–6.0) was collected from a local water reclamation plant (Singapore) with total solids (TS) of 25 ± 2 g TS L^{-1} and volatile solids (VS) of 20 ± 2 g VS L^{-1}. Alkaline treatment of feed sludge was conducted in a 1.5 L batch reactor. The raw feed sludge was adjusted to pH 9 with 5 N NaOH, and then stirred at 200 rpm for 1 h (h), which covered the optimum sludge solubilisation duration (Li et al., 2008). 1 L nitrogen sparged alkaline pre-treated sludge was fed daily into a 5 L fermenter (BIOSTAT Aplus MO, Satorius, Germany) operated in semi-continuous and completely mixing mode. The fermenter was initially inoculated with 3 L seed anaerobic sludge (volatile suspended solids (VSS) = 9.12 g L^{-1}) collected from an acidogenic reactor (HRT of 3 d and pH controlled at 7.5) of a laboratory-scale three-stage anaerobic digestion system. The semi-continuous fermenter was operated at a HRT of 3 days (d) (HRT equals to SRT due to complete mixed mode) with stirrer speed at 250 rpm and temperature of 35 °C. The pH in the fermenter was self-adjusted and in the range of 6.2–8.1.

Different salinity levels were set to investigate salinity effects on VFAs production and the fermenter operation comprised five periods: (1) Acclimation (0th – 24th d) to acclimate to the new feedstock and operating mode; (2) Phase I (25th – 55th d) with 3 parts per thousand (‰) salinity in the fermenter, as this is the initial salinity concentration in the acidogenic reactor before adding sodium chloride (NaCl); (3) Phase II (56th – 63rd d) with salinity increased to 10‰; (4) Phase III (64th – 103rd d) with salinity increased to 20‰ and (5) Phase IV (104th – 112nd d) with salinity increased to 40‰. Salinity in the fermenter was controlled by manipulating NaCl in the alkaline pre-treated feed sludge (Supplementary data: Fig. S1). Sludge samples were drawn daily from the fermenter for VFAs, COD, VS, and biogas analysis.