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Effects of magnesium chloride on the anaerobic digestion and the implication on forward osmosis membrane bioreactor for sludge anaerobic digestion

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ABSTRACT

This work elucidates the effects of model reversed salt $MgCl_2$ on methane production in an anaerobic digestion bioreactor treating waste sludge. Along with $MgCl_2$ concentration being raised stepwise, the methane production was only slightly less than in the control when $MgCl_2$ was 20 g/L and under, and then suddenly reduced to only about 10 mL/(L·d) at a $MgCl_2$ concentration of 30 g/L, and finally stopped when the $MgCl_2$ concentration reached 50 g/L. However, the total relative abundance of methanogens *Methanomicrobia* and *Methanobacteria* still accounted for 84.97% of the archaeal community when $MgCl_2$ was 50 g/L. The high correlation between live/dead cell ratio and methane production suggests that the live/dead cell ratio instead of the inhibition of methanogen might be the major cause for the halt of methane production at a magnesium chloride concentration of 50 g/L.

1. 1. Introduction

Anaerobic digestion (AD) attracts much interest among researchers globally since it can achieve sludge stabilization by converting part of its organic matter into biogas – a renewable energy source. Applications of MBRs in sludge digestion have been studied and practiced (Wang et al., 2008). However, it is difficult to employ low pressure-driven membranes for digested sludge filtration, because the composition of digested sludge is so complex that it would foul low pressure-driven membranes quickly and easily. As a result of the dense membrane surface of forward osmosis (FO) membranes and very low pressure applied in the FO process, fouling of FO membranes might be much less than pressure-driven membranes. It has been repeatedly reported that membrane fouling and flux declines were lower in forward osmosis membrane bioreactor (FO-MBR) (Cornelissen et al., 2008; Lutchmiah et al., 2014; Wang et al., 2014). Therefore, forward osmosis has been preliminarily studied for application in aerobic sludge digestion (Zhu et al., 2012) and in anaerobic sludge digestion (Li et al., 2017).

The performance of AD process could be largely affected by the type of feed substrates, i.e. agricultural wastes, food wastes, or sewage sludge (Chen et al., 2008). Since glucose was the only substrate that has been studied in reports of FO related anaerobic digestion (Kim et al., 2016; Li et al., 2017), it is quite pressing and exciting to examine the performance of this process in treating real waste sludge.

Another factor that could influence AD is the draw solution employed in FO process, which is mainly inorganic salt. Reverse salt flux (RSF) from draw solution side to digested sludge side during FO-MBR operation might be a thorny problem for FO digestion process. Due to limitations of FO membranes, higher water flux means higher RSF in most cases. Therefore, the accumulation of salt in sludge would probably influence the performance of MBR. There were several reports about salt effects on aerobic MBR. The effects of gradual increase of salt concentration (0–35 g-NaCl/L) on the performance of MBR were studied in aerobic process (Johir et al., 2013). When NaCl shock loading increased from 5 to 60 g/L, total Kjehldahl nitrogen removal efficiency was observed to drop from 95% to 23% (Yogalakshmi and Joseph, 2010).

Compared to the aerobic process, the anaerobic process is more vulnerable to external environmental changes such as the salt concentration. Therefore, RSF might have an even greater impact on anaerobic digestion FO-MBR (adFO-MBR). Previously published studies have demonstrated that the presence of salt influenced the performance of anaerobic digestion on sludge. It was reported that 3.5–5.5 g/L Na^+ in sludge caused moderate inhibition on the activities of methanogens,

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and 8.0 g/L Na^+ seriously inhibited the production of methane (Chen et al., 2008). Sheng Li studied the impact of RSF on microbial community and bio-methane production in an adFO-MBR system for 20 days. The adFO-MBR system was driven by simulated fertilizers, i.e. three types of K^+ salt as draw solutes. The impact of RSF on methane production was found to be through changes of bacterial community rather than archaeal community in the adFO-MBR. The lowest methane production (less than 65 mL) was observed when the fertilizer was KNO_3 and RSF of KNO_3 was 12.2 g/m^2/h (Li et al., 2017).

Compared with monovalent ions like Na^+ and K^+ mentioned above, divalent draw solutions, such as $MgSO_4$, $MgCl_2$, and $CaCl_2$, which are larger-sized cations could lead to lower RSF even for the same FO membrane (Achilli et al., 2010). Among divalent ions, $MgCl_2$ was considered to be one of the best FO draw solutions for most water and wastewater applications and should be further investigated for environmental engineering applications, because most FO applications using $MgCl_2$ as draw solution have relatively higher water flux, lower RSF and no risk of scaling (Achilli et al., 2010).

As one kind of macronutrients, magnesium is required for the activation or function of many microorganisms. Therefore, magnesium within proper concentration range could enhance AD performance to some extent. It was also frequently used as a struvite precursor to remove excess ammonia and phosphate (Romero-Güiza, et al., 2015). Previous reports also indicated that the process of Mg^{2+} inhibition was a progressive one rather than a steep one (Romero-Güiza et al., 2016). The threshold of inhibition concentration of Mg^{2+} was higher than that of K^+ (Syazwani et al., 2017). Therefore, $MgCl_2$ should be a promising candidate for the best draw solution for adFO-MBR process.

As stated above, we still lack understanding of how RSF (specifically Mg^{2+}) in the FO process affects anaerobic digestion step-by-step during long term operation, especially when real waste sludge is used as the substrate. To answer this question in this study, we examined the effects of RSF on removal rate of organics, methane and VFAs production, and microbial components in two adFO-MBRs. Additionally, the correlation between RSF and microbial components was also analyzed.

2. Materials and methods

2.1. Feed sludge and inoculated sludge

Dewatered sewage sludge from YongFeng wastewater treat plant (Beijing, China) was used as the feed substrate for anaerobic digestion (AD) reactors. The total solids (TS) of dewatered sludge ranged from 20% to 23% (w/w) and volatile solids (VS) accounted for 50–51% of TS. The substrate sludge was stored at 4 °C and dissolved into deionized water (DI) before everyday feeding to make the TS around 6%. The mesophilic seed sludge collected from an anaerobic digester at an ecological garden (Beijing, China) had TS of 2.6% (w/w), of which VS made up 56.8%.

2.2. FO experiments for RSF determination

To determine the specific amount of $MgCl_2$ to be added into the simulated adFO-MBR digester, FO experiments were conducted to evaluate the RSF of $MgCl_2$ at different concentrations, i.e. 47.61 g/L (0.5 mol/L), 95.21 g/L (1.0 mol/L), 190.42 g/L (2.0 mol/L), 380.84 g/L (4.0 mol/L). The thin film composite (TFC) FO membrane used for evaluation was fabricated in the laboratory, and the preparation method is presented in supplementary materials. The evaluation experiment lasted for 20 h, and the initial volume of draw solution (DS) was 1000 mL for all four tested $MgCl_2$ solution. The highest detected RSF of $MgCl_2$ was 32.9 ± 1.0 g/m^2/h, when the concentration of $MgCl_2$ was 380.84 g/L (4.0 mol/L). To simulate a long-time operation for adFO-MBR, a maximum $MgCl_2$ concentration of 50 g/L in digester sludge was chosen for evaluation.

2.3. Experimental protocol

Two conventional sludge anaerobic digestion reactors were operated continuously for 122 d with a constant hydraulic retention time (20 d) in semi-continuous mode. One of these two reactors (Mg-digester) was used for $MgCl_2$ tests, and the other one was used as control. The working volumes of both reactors were 400 mL. We fed 20 mL sludge into each of the digesters and then discharged the same amount from both of the digesters daily. The corresponding ratio of feed sludge to inoculated sludge was 1:1. Digesters were maintained at a mesophilic temperature (37 °C) by being kept in thermostatically heated water bath (Scientz Biotechnology CO., LTD, Ningbo China). The headspace of each digester was purged with nitrogen gas for 3 min. Both digesters were tightly closed with blue cap bottles and manually shaken for 1 min twice a day. The operation of the Mg-digester was separated into 12 successive phases according to the $MgCl_2$ concentration, which was increased from 1 g/L in the first phase to 50 g/L in the 12th phase stepwise but kept at the same level during each phase. Certain amount of $MgCl_2$ was added into the daily feed sludge to keep the concentration of $MgCl_2$ constant during each phase. When one phase was over, certain amount of $MgCl_2$ was dosed into the reactor directly according to the set value of the next phase. In the control reactor, all conditions except Mg concentration were kept the same through all the operation. Both of the digesters had an organic load of 1.0 ± 0.1 gVS/L/d.

2.4. Chemical analytical methods

Conductivity and pH of the AD sludge were measured using a conductivity meter (DDSJ-318, Inesa, shanghai, China) and a pH meter (MP522, Sanxin, China) respectively. TS and VS of AD sludge were measured with the weight difference method. The COD and NH_4-N concentrations were determined by a fast water quality analyzer (Lianhua, China).

Volatile fatty Acids (VFAs) of AD sludge was analyzed by a GC (6890N, Agilent, USA) with flame ionization detector. To analyze VFAs, sludge samples taken from the reactors were centrifuged first at 10,000 rpm for 10 min. Then the supernatant was passed through a microfiber filter (0.45 μm). Biogas composition (CH_4 and CO_2) was determined using an Agilent Technologies 7890B GC system.

2.5. Live/dead cells analysis

The live and dead cells in the digesters which might be affected by $MgCl_2$ were detected by confocal laser scanning microscopy (CLSM) using the LIVE/ DEAD® BacLight™ (Invitrogen, USA). The anaerobic sludge was stained with SYTO® 9 and propidium iodide (PI) according to reagent manual (LIVE/ DEAD® BacLight™, Invitrogen, USA). The Auto-PHLIP-ML and Image J softwares were used to analyze the proportion of the live/dead bacteria in the Mg-digester.

2.6. Microbial community analysis

The microorganism community structure was determined by using the high-throughput sequencing method (Lei et al., 2016). We collected 10 mL sludge samples when the Mg-digester were steady in each phase and then kept the samples stored below −20 °C before delivering to Allwegene Co., Ltd. (Beijing, China) for high-throughput sequencing. DNA was extracted from sludge samples from different phases of $MgCl_2$ concentration, i.e.2 g/L, 8 g/L, 30 g/L, 50 g/L, via PowerSoil® DNA Isolation Kit (Mobio Laboratories, Inc., USA) according to the manufacturer's instructions. After extraction of genomic DNA, electrophoresis (1% agarose gel) was used to detect genomic DNA extraction.

Then archaeal and bacterial 16S rRNA gene fragments were amplified via the polymerase chain reaction (PCR) with the primer sets of Arch344/Arch806 and 338F/806R, respectively (Liu, et al., 2016; Yin, et al., 2018). The quality of the PCR product was determined with 2%