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Short Communication

Enhancing blackwater methane production by enriching hydrogenotrophic methanogens through hydrogen supplementation

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ABSTRACT

Source diverted blackwater (i.e., toilet wastewater with or without food waste residuals) represents an ideal waste stream for bioenergy recovery through anaerobic digestion. However, due to the high free ammonia concentration contained in blackwater collected from water conserving toilets (e.g., vacuum toilets), CH_4 recovery can be significantly reduced because methanogenesis is inhibited. Our current study evaluated the feasibility of enriching hydrogenotrophic methanogens (known to be more resistant than acetoclastic methanogens toward free ammonia inhibition) in anaerobic sludge with H_2 supplementation for blackwater treatment. Our results demonstrated that hydrogenotrophic methanogens can be enriched by supplementing anaerobic sludge with H_2 and CO_2 in a volume ratio of 4:1 for 3 months. The dominance of hydrogenotrophic methanogens after 3-month enrichment was confirmed with quantitative PCR studies. It was observed that the hydrogenotrophic methanogen dominant anaerobic sludge significantly alleviated blackwater free ammonia inhibition, and led to enhanced biochemical methane production (BMP) (e.g., from 30% to 53%). Our current study offers a new treatment option for anaerobic treatment of wastewater with high free ammonia content.

1. Introduction

Worldwide water and energy scarcity has triggered great efforts on the development of energy efficient wastewater treatment technology (Shao et al., 2018; Xu et al., 2018b; Yi et al., 2019). New decentralized wastewater treatment can be designed and developed based on source diversion of household wastewater streams such as blackwater (toilet water plus food waste residuals), from all other household wastewater (greywater) treated locally, to maximize energy, nutrient, and water recovery. Source diverted blackwater contains high organic and nutrient levels and is an ideal waste stream for wastewater bioenergy recovery through anaerobic digestion (AD). Blackwater characteristics vary significantly based on the amount of water used for toilet flushing. For instance, vacuum toilets use a minimal amount of flushing water and generate blackwater with high organic and nutrient content, while blackwater from dual-flush toilets and conventional toilets (with no water saving options) will typically be diluted 2–10 fold compared to blackwater from vacuum toilets (Florentino et al., 2019; Gao et al., 2019). Our previous studies demonstrated that the high ammonia concentration—ammonium + free ammonia (FA)— in blackwater collected from water saving toilets (e.g., vacuum toilets) can result in severe inhibition of methanogenesis and subsequently lead to a low biochemical methane potential (BMP) (Gao et al., 2019). Free molecule of NH_3 and the ion NH_4^+ are two most significant methanogenesis inhibitors when present in high concentrations in anaerobic digestion processes (Fotidis et al., 2014). The inhibition of methanogenesis occurs because NH_3 and NH_4^+ can interact or directly diffuse into cells and lead to changes in intracellular pH, depletion of the intracellular potassium, or/and inhibition of specific enzyme reactions (Fotidis et al., 2014). As a result, the anaerobic digestion process may deteriorate, or run stably but with a low methane yield (Chen et al., 2008).

Methanogenic archaea are more vulnerable to ammonia toxicity than other groups of microorganisms involved in AD (Wang et al., 2016). Two distinct methanogenic archaea groups participate in methanogenesis: (i) versatile/strictly acetoclastic methanogens (e.g., *Methanosarcinaceae/Methanosaetaceae*) and (ii) strictly hydrogenotrophic methanogens (e.g., *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales*, *Methanocellales*, and *Methanopyrales*) (Fotidis et al., 2014; Sarmiento et al., 2011). Acetoclastic methanogens are dominant over hydrogenotrophic methanogens in conventional AD because biological H_2 production is not an energy favorable pathway in AD (Liu et al., 2016; Xu et al., 2018a). However, acetoclastic methanogens are more

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sensitive than hydrogenotrophic methanogens to ammonia toxicity (Fotidis et al., 2014; Luo et al., 2012). It has been reported that when the ammonium concentration is high, methane production shifts from acetoclastic methanogens to hydrogenotrophic methanogens (Westerholm et al., 2012).

Options have been evaluated for switching the dominance of acetoclastic methanogens to hydrogenotrophic methanogens in AD systems. Pure cultured hydrogenotrophic methanogens (*Methanoculleus* sp.) may be supplied in AD systems through bioaugumentation (Lebiocka et al., 2018). However, maintaining the bioaugumented hydrogenotrophic methanogens in AD systems is a challenge as after short improvement period, bioaugmented microbes often get outcompeted by indigenous organisms (Herrero & Stuckey, 2015). Electrochemical based microbial processes, such as micobial electrolysis cells (MECs), can cultivate a hydrogenotrophic methanogens dominant biomass in the cathode chamber, although complex reactor setup and system maintenance are needed (Cerrillo et al., 2017). One attractive option is to supply H_2, either directly to an existing AD reactor or to a side stream sludge selector to enrich hydrogenotrophic methanogens (Hao et al., 2017). However, when H_2 is directly supplied to an AD reactor, elevated H_2 partial pressure (P_{H2}) may inhibit volatile fatty acid (VFA) (mainly propionate and butyrate) fermentation; thus the process must be carefully controlled (Luo et al., 2012). H_2 addition to a side stream sludge selector and return of the sludge to the AD reactor mitigate the inhibition of high H_2 partial pressure in AD and could maintain the dominance of hydrogenotrophic methanogens in AD sludge. Few studies have reported the application of H_2 for hydrogenotrophic methanogen enrichment (a search on the Web of Science database revealed four publications). These studies did not report the impact of H_2 supplementation on hydrogenotrophic methanogen and acetoclastic methanogen microbial community changes, nor the impact of these changes on AD performance in the presence of free ammonia inhibition.

In the present study we added H_2 to a side stream sludge selector to enrich hydrogenotrophic methanogens, then applied the sludge treated with H_2 to the treatment of blackwater collected from different toilets (with various levels of organics and free ammonia concentrations). Methanogen groups often found to dominate anaerobic digesters (four hydrogenotrophic methanogens: *Methanomicrobiales*, *Methanobacteriales*, *Methanoculleus*, *Methanothermobacter*, and two acetoclastic methanogens: *Methanosaeta*, *Methanosarcina*) were quantified using the quantitative polymerase chain reaction (qPCR). This research represents the first attempt to enhance blackwater treatment by manipulating the abundance of acetoclastic methanogens and hydrogenotrophic methanogens.

2. Materials and methods

2.1. Blackwater type and source

Blackwater was collected and treated separately in this research. Blackwater stock (urine and feces) with no flush water was collected from the University of Alberta campus. Based on both water-conserving (0.5–1 L) and water-wasting (5–9 L) toilet flush options, tap water was used to dilute the blackwater stock to 0.5 L (representing vacuum toilets), 1 L (representing vacuum toilets), 5 L (representing dual flush toilets), and 9 L (representing conventional toilets) water-flush configuration; these samples were termed 0.5 L blackwater, 1 L blackwater, 5 L blackwater and 9 L blackwater, respectively. Blackwater samples were stored anaerobically at 4 °C for a maximum of seven days before being introduced to the reactors.

2.2. Enrichment of hydrogenotrophic methanogens through H_2 supplementation

The enrichment of hydrogenotrophic methanogens was performed by directly injecting H_2 to serum bottles (2 L volume each) seeded with anaerobic sludge obtained from a full-scale mesophilic anaerobic digester treating waste activated sludge in Alberta. The bottles were sealed with butyl rubber stoppers and aluminum crimps after 1 L of inoculum anaerobic sludge was introduced. The bottles were incubated in a shaker at a shaking speed of 120 rpm and a temperature of 35 °C. The bottles were supplemented every three days for three months with 400 mL H_2 and 100 mL CO_2 (in a mixed gas of H_2:CO_2 = 4:1 in volume ratio, pure standard) by directly injecting (using the needle of a tube filled with gas) the gas mixture to the bottom of the bottles. pH, total ammonia nitrogen (TAN) concentration and total COD (COD_t) concentration of enriched anaerobic sludge were measured in triplicate when the enrichment process was complete.

2.3. Blackwater treatment with H_2 enriched anaerobic sludge

The enriched anaerobic sludge from the serum bottles was collected to treat the blackwater from different toilet types (i.e., 0.5 L blackwater, 1 L blackwater, 5 L blackwater and 9 L blackwater) using 160 mL serum bottles. New serum bottles were seeded with 50 mL enriched anaerobic sludge as inoculum and 50 mL blackwater as the feed. To establish anaerobic conditions, the head space was flushed with N_2 gas (99.99% in purity) for at least 5 min before sealing the serum bottles with butyl rubber stoppers (Bellco Gass, Vineland, NJ) and crimp caps. No H_2 was injected in this experiment. Parallel bottles inoculated with blackwater and original anaerobic sludge (without H_2 enrichment) served as controls. All experiments were conducted at 35 °C and concluded when biogas production ceased (around 40 days). CH_4 production and COD reduction rates in the blackwater were monitored, and blackwater BMP was calculated.

2.4. Analysis of methanogen communities

Four hydrogenotrophic methanogens (*Methanomicrobiales*, *Methanobacteriales*, *Methanoculleus*, *Methanothermobacter*) and two acetoclastic methanogens (*Methanosaeta*, *Methanosarcina*) were quantified using quantitative PCR. Quantitative PCR was applied by targeting 16S rRNA genes of methanogens from two treatment groups: (1) original anaerobic sludge vs. H2 enriched anaerobic sludge, and (2) H_2 enriched anaerobic sludge before vs. after 1 L blackwater AD treatment. Genomic DNA was extracted from a 1 mL sample of mixed liquor taken directly from anaerobic reactors using a QIAGEN PowerSoil Pro Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The concentration and purity of extracted DNA were analyzed by NanoDrop (NanoDrop One, Thermo Scientific, Walthan, MA) and agarose gel electrophoresis (1%). Concentrations of DNA were approximately 50 ng μL^{-1}, with 260/280 ratios of approximately 1.8.

All primers were synthesized by Integrated DNA Technologies (Iowa, USA). The quantitative PCR amplifications were performed using a CFX96TM Real-Time Detection System (Bio-RAD, California, USA) according to previous research (Huang et al., 2016). Briefly, 20 µL of each reaction was performed in 96-well microplates. The following two-step conditions was performed: one initial enzyme activation step of 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 20 s and simultaneous annealing/extension at 60–68 °C for 20 s. The 20 µL qPCR mix cocktail contained 1 µL template, 10 µL SsoFas EvaGreen supermix (Bio-Rad), 0.4 µL of each primer (20 µM) and DNase-free water added to the final volume. The 50 µL end-point PCR cocktail (GoTaq PCR Core system I) contained 1 µL template, 3 µL Mg^{2+} in magnesium chloride solution, 10 µL Colorless GoTaq Flexi Buffer (5X), 1 µL PCR nucleotide mix, 1 µL of each primer (20 µM), 0.25 µL GoTaq DNA polymerase and DNase-free water added to the final volume. The end-point PCR amplifications were performed in Bio-Rad thermal cycle with one initial denaturation at 95 °C for 5 min; 30–35 cycles of denaturation at 95 °C for 45 s, annealing at 51–58 °C for 45–90 s, along with extension at 72 °C for 45–60 s; and final extension at 72 °C for 10 min and incubated at 10 °C.

Gene copy numbers were calculated by comparing threshold cycle values obtained in each PCR run with those of known plasmid DNA

standard concentrations. Standard curves were constructed using a purified DNA template that was amplified by conventional PCR using the corresponding primers. All experiments were performed in triplicate for each sample; all PCR runs included control reactions without the DNA template.

2.5. Chemical and statistical analyses

The composition of biogas (mainly CH_4, CO_2, and H_2) in the headspace of the serum bottles was measured with a gas chromatograph (GC-7890B, Agilent Technologies, Santa Clara, CA) equipped with a G3591-81023, a Hayesep Q column, and a flame ionization detector. Helium (99.999%) was used as carrier gas. Biogas volume was recorded daily with a gas meter. The accumulated $CH_4/CO_2/H_2$ was obtained by multiplying biogas volume and biogas fraction measured by gas chromatography. All results were obtained at standard temperature and pressure. pH was measured immediately after sampling using a Symphony pH probe (VWR, Radnor, PA). Biomass concentration was measured in COD units (Xu et al., 2014). Water quality of blackwater—COD, soluble COD (CODs), TAN, total phosphorus (TP), and total suspended solids (TSS)—were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2002). The BMP was calculated as a percentage of the influent COD converted to methane. Free ammonia (FA) was calculated with Eq. (1).

$$FA = S_{(NH_4^+-N+NH3-N)} \times 10^{pH}/(K_b/K_w + 10^{pH})) \quad (1)$$

where the K_b/K_w was determined via $K_b/K_w = e^{6344/(273+T)}$, $S_{(NH4\text{-}N+NH3\text{-}N)}^{+}$ is the total ammonia concentration, and T is the operation temperature (35 °C in our study).

One-way ANOVA analysis was performed to assess significant differences among groups, with p values < 0.05 indicating statistical significance.

3. Results and discussion

3.1. Enrichment of hydrogenotrophic methanogens

H_2 enrichment of hydrogenotrophic methanogens was conducted over three months with the direct injection of H_2 (0.406 ± 0.07 mL/mL inoculum) and CO_2 (0.106 ± 0.007 mL/mL inoculum) in a volume ratio of 4:1 to serum bottles seeded with anaerobic sludge obtained from a full-scale mesophilic anaerobic digester treating waste activated sludge every three days for three months. When exogenous H_2 and CO_2 is supplemented in mesophilic AD, H_2 consumption pathways potentially consist of two pathways and three groups of microorganisms. In the first pathway, H_2 may be consumed by hydrogenotrophic methanogens (pathway I, Eq. (2)) through simultaneous consumption of CO_2 and H_2 at a molar ratio (i.e., volume ratio) of 4:1, and the production of CH_4. In the second pathway, homoacetogens (H_2-consuming bacteria) can consume H_2 and CO_2 at a molar ratio of 2:1 to produce CH_3COO^-, which can then be utilized by acetoclastic methanogens for CH_4 production (pathway II, Eqs. (3) and (4)) (Liu et al., 2016). It should be noted that in a conventional AD process, homoacetogens are highly limited (i.e., contributing 2–5% of H_2 consumption) due to the low H_2 partial pressure (P_{H2}). Further, compared to the hydrogenotrophic methanogen pathway for H_2 consumption, the second pathway, which depends on the synergic effect of homoacetogens and acetoclastic methanogens, is thermodynamically less favorable as more energy is required for cell maintenance and growth (Deppenmeier et al., 1996).

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O; \quad \Delta G^0 = -131 \text{ kJ/mol; } \textit{hydrogenotrophic methanogens} \quad (2)$$

$$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O; \quad \Delta G^0 = -95 \text{ kJ/mol; } \textit{homoacetogens} \quad (3)$$

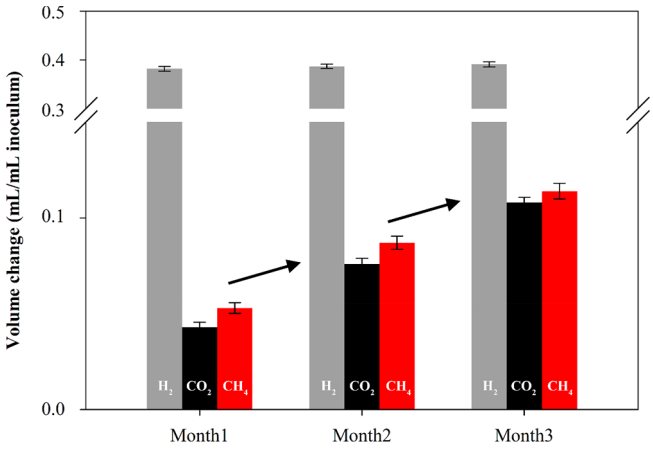

Fig. 1. Changes in H_2 consumption volume (grey), CO_2 consumption volume (black) and CH_4 production volume (red) during the three months enrichment of hydrogenotrophic methanogens. Error bars represent one standard deviation of the mean from triplicate experiments. Ratios of volume were also provided. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4; \quad \Delta G^0 = -31 \text{ kJ/mol; } \textit{acetoclastic methanogens} \quad (4)$$

Fig. 1 shows the reduction volume of H_2 and CO_2 and the production volume of CH_4 at the end of each month. After the first month of enrichment, 0.382 ± 0.005 mL/mL inoculum (94%) of H_2 and 0.043 ± 0.003 mL/mL inoculum (40%) of CO_2 were consumed, respectively, with a total of 0.053 ± 0.003 mL CH_4/mL inoculum produced, comprising an approximate volume ratio of 8:1:1 (H_2:CO_2:CH_4). Considering the two major potential H_2 consumption pathways, the high H_2 to CO_2 consumption ratio (8:1, pathway I 4:1, and pathway II 2:1) observed may be attributed to the presence of other inorganic electron acceptors (e.g., nitrate, ferric iron, sulfate) that can also consume H_2 (e.g., Eq. (5)) in the freshly seeded inoculum (Tang et al., 2012).

$$NO_3^- + 3.03H_2 + 0.23CO_2 + H^+ \rightarrow 0.48N_2 + 0.046C_5H_7O_2N + 3.37H_2O \quad (5)$$

At the end of the second month, the reduction of H_2 and CO_2 was 0.387 ± 0.004 mL/mL inoculum and 0.076 ± 0.003 mL/mL inoculum, respectively, and the production of CH_4 increased to 0.087 ± 0.005 mL/mL inoculum per cycle, with an H_2:CO_2:CH_4 volume ratio of 5:1:1. Compared to the first month of hydrogenotrophic methanogen enrichment, a similar amount of H_2 (95% vs 94%) was removed from the exogenous H_2 source but more CO_2 (72% vs 40%) was converted to CH_4, suggesting that hydrogenotrophic methanogens became predominant gradually (Eq. (2)). At the end of the third month, with a consistent H_2 reduction volume of 0.391 ± 0.005 mL/mL inoculum, the consumption of CO_2 and the production of CH_4 further increased to 0.108 ± 0.003 mL/mL inoculum and 0.114 ± 0.004 mL/mL inoculum, respectively, indicating that the long-term enrichment increased the CO_2 consumption rate. Finally, the volume change ratio of H_2:CO_2:CH_4 reached 4:1:1 and the injected H_2 and CO_2 were completely consumed following the stochiometric ratio of hydrogenotrophic methanogenesis, indicating that the enrichment process was complete, and that the enriched hydrogenotrophic methanogens were dominant.

Quantitative PCR was used to obtain direct evidence for hydrogenotrophic methanogen enrichment. Fig. 2 shows that the methanogenic 16S rRNA gene abundance changed before and after enrichment. The average gene abundances of hydrogenotrophic methanogens *Methanomicrobiales, Methanobacteriales, Methanoculleus,* and *Methanothermobacter* were 1.05×10^5, 2.09×10^5, 9.0×10^3, and

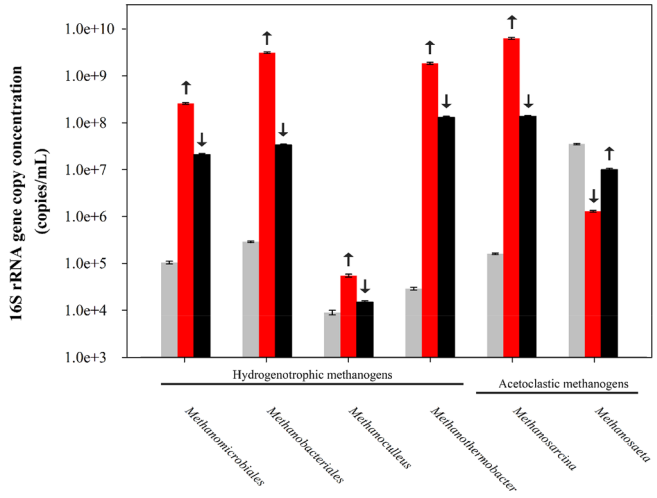

Fig. 2. Changes in 16S rRNA gene copy abundance of methanogenic population before (grey) and after (red) 3 months H_2 enrichment, and after 40 day 1 L blackwater AD treatment (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

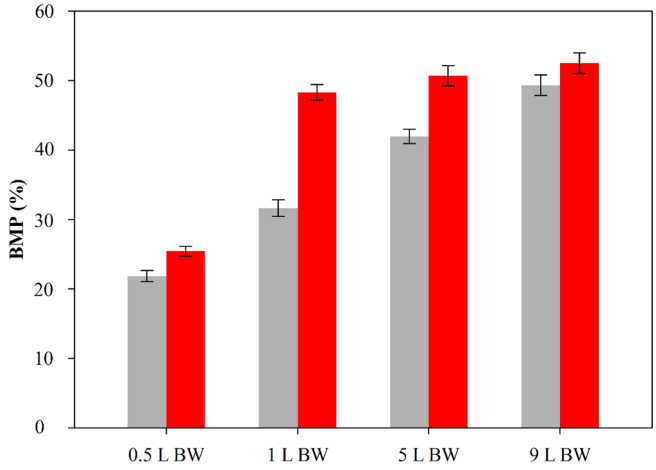

Fig. 3. Change in BMP after the 40 day blackwater AD treatment seeded with original anaerobic sludge (grey) and enriched anaerobic sludge (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.9×10^4 copies/mL, respectively, in the original anaerobic sludge. After 3 months of H_2 supplementation, the gene abundances of the hydrogenotrophic methanogens significantly increased to 2.62×10^8,3.19×10^9, 5.53×10^4, and 1.88×10^9 copies/mL, respectively ($p < 0.05$ for all). The gene abundance of *Methanosarcina* (a versatile acetoclastic methanogen), which can consume both H_2 and acetate, also increased significantly from 1.61×10^5 to 6.39×10^9 copies/mL ($p < 0.05$) after three months of exogenous H_2 supplementation. In contrast, the gene abundance of *Methanosaeta* (strictly acetoclastic methanogens) decreased significantly from 3.55×10^7 to 1.3×10^6 copies/mL ($p < 0.05$) as no carbon and energy source (i.e., acetate) was supplied. Our results showed that hydrogenotrophic methanogens became the dominant methanogens after H_2 supplementation.

3.2. Blackwater treatment using hydrogenotrophic methanogens in enriched anaerobic sludge

Both enriched and original anaerobic sludge were applied in blackwater sample (0.5 L, 1 L, 5 L, and 9 L) treatment for comparison. Blackwater characteristics are listed in Table 1. Briefly, the total COD (COD_t) concentrations of blackwater samples 0.5 L, 1 L, 5 L, and 9 L were 36.40 ± 2.09, 18.03 ± 0.25, 3.83 ± 0.22, and 2.28 ± 0.10 g/L, respectively, at pH 8.44 ± 0.09, 7.97 ± 0.11, 7.88 ± 0.05, and 8 ± 0.07, respectively, and a total ammonia concentration of 1675 ± 19, 926.7 ± 14, 182.7 ± 2.7, and 102.3 ± 2.4 mg/L, respectively, which resulted in FA concentrations of 398.7, 88.7, 14.5, and 10.4 mg/L, for 0.5 L, 1 L, 5 L, and 9 L flushed blackwater samples, respectively, according to Eq. (1).

Blackwater biochemical methane production (BMP) values are presented in Fig. 3. As shown in Fig. 3, in the original anaerobic sludge, the most pronounced ($p < 0.05$) inhibition was observed in 0.5 L flushed blackwater sample due to the highest FA concentration, which resulted in a low BMP of 22%. BMP increased gradually to 49% in blackwater with low FA concentration (i.e., 9 L blackwater). This observation is in agreement with previous studies that blackwater FA concentrations greater than 205 mg/L leads to significant BMP inhibition (Florentino et al., 2019; Gao et al., 2019).

It has been reported that under FA inhibition, methane production from acetate can shift from acetoclastic methanogenesis to syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (Chen et al., 2016; Yin et al., 2018). As shown in Fig. 3, pronounced alleviation of FA inhibition by using enriched anaerobic sludge was observed only in the treatment of 1 L and 5 L flushed blackwater samples, in which the BMP was enhanced from 32 ± 1% to 48 ± 11% ($p = 0.04$) and from 42 ± 1% to 53 ± 1% ($p = 0.045$), respectively (Fig. 3). When FA was reduced to 10.4 mg/L (9 L flushed blackwater sample), enhancement of the BMP by enriched anaerobic sludge became insignificant (6.4%) ($P = 0.4$), which was probably due to an insignificant FA inhibition at low FA (e.g., 17–108 mg/L) (Gao et al., 2019) in the original anaerobic sludge in which BMP reached almost maximum capacity. In 0.5 L flushed blackwater samples, although BMP was enhanced slightly from 22 ± 1% to 25 ± 1% ($P = 0.046$) in the H_2 enriched anaerobic sludge, FA inhibition was not overcome by a hydrogenotrophic methanogen dominant culture. A previous study also demonstrated that hydrogenotrophic methanogen communities can be inhibited at high ammonium concentrations (e.g., > 5 g/L NH_4^+-N) (Wang et al., 2016).

3.3. Decay of enriched hydrogenotrophic methanogens

The reduction in the gene abundance of hydrogenotrophic methanogens in the enriched anaerobic sludge was observed in 1 L flushed blackwater samples (Fig. 2). After blackwater was fed as a substrate, the gene abundance of hydrogenotrophic methanogens *Methanomicrobiales,*

Table 1
Characteristics of inoculum and blackwater.

Parameters	pH	COD_t (g/L)	CODs (g/L)	TAN (mg/L)	FA (mg/L)	TP (mg/L)	TSS (mg/L)
Original anaerobic sludge	7.33 ± 0.02	7.48 ± 0.55	/	1205.3 ± 17	/	/	/
Enriched anaerobic sludge	8.09 ± 0.03	7.88 ± 0.28	/	1069.3 ± 23	/	/	/
0.5 L blackwater	8.44 ± 0.09	36.40 ± 2.09	10.96 ± 0.23	1675 ± 19	398.7	590 ± 15	32860 ± 324
1 L blackwater	7.97 ± 0.11	18.03 ± 0.25	5.64 ± 0.10	926.7 ± 14	88.7	330 ± 11	17140 ± 104
5 L blackwater	7.88 ± 0.05	3.83 ± 0.22	1.35 ± 0.09	182.7 ± 2.7	14.5	69 ± 4	3970 ± 108
9 L blackwater	8 ± 0.07	2.28 ± 0.10	0.71 ± 0.05	102.3 ± 2.4	10.4	38 ± 5	2390 ± 105

Methanobacteriales, Methanoculleus, and *Methanothermobacter* slightly decreased ($p < 0.05$) from 2.62×10^8, 3.19×10^9, 5.53×10^4, and 1.88×10^9 copies/mL to 2.1×10^7, 3.4×10^7, 1.5×10^4, and 1.3×10^8 copies/mL, respectively. On the other hand, the gene abundance of *Methanosaeta* (a strictly acetoclastic methanogen) increased from 1.3×10^6 to 1.0×10^7 copies/mL ($p < 0.05$). The gene abundance of *Methanosarcina* (a versatile acetoclastic methanogen), which is considered to be robust to ammonia inhibition due to its spherical cells (Gao et al., 2019), also decreased slightly ($p < 0.05$) from 6.4×10^9 to 1.4×10^8 copies/mL when blackwater was used as a substrate. Our study reported for the first time the enriched hydrogenotrophic methanogen microbial community changes after AD process in the presence of free ammonia inhibition. Future studies on the stability of an enriched hydrogenotrophic methanogen culture over long-term operation of continuous operating digesters are needed to further evaluate the feasibility of H_2 enrichment technologies.

4. Conclusion

We propose a new approach to blackwater treatment by using exogenous H_2 supplementation to enrich hydrogenotrophic methanogens. After three months of hydrogenotrophic methanogen enrichment, the free ammonia inhibition of methanogenesis in blackwater collected from water conserving toilets was alleviated, as shown in enhanced methane production. However, reduction in the 16S rRNA gene abundance of the enriched hydrogenotrophic methanogen culture was observed during the 40 day blackwater treatment. Further studies are encouraged to investigate the stability of an enriched hydrogenotrophic methanogen culture over long-term operation of continuous operating digesters, and the replacement of exogenous H_2 with economical and green sources.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.01.014.

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