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A long-term study on the effect of magnetite supplementation in continuous anaerobic digestion of dairy effluent – Enhancement in process performance and stability

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HIGHLIGHTS

- A long-term study on the effect of magnetite in continuous biomethanation was made.
- Magnetite supplementation was beneficial to the continuous biomethanation of whey.
- Methane production and process stability were improved with magnetite addition.
- DIET via conductive magnetite likely contributed to the enhanced biomethanation.
- *Methanosaeta* was likely involved in DIET as well as aceticlastic methanogenesis.

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ABSTRACT

Interspecies electron transfer (IET) between microbial populations with different functions is critical to stable anaerobic digestion. This study, in an attempt to facilitate IET, investigated the effect of magnetite supplementation on the biomethanation of dairy effluent in continuous mode. The magnetite-added reactor (RM) was significantly more resistant and resilient to process imbalance than the reactor run without magnetite addition (RC). RC showed unstable performance with repeated process upsets, but its performance improved to be comparable to that of RM after applying magnetite supplementation. Magnetite was particularly effective in stabilizing a build-up of propionic acid and therefore improving the process robustness and reliability. The enhanced biomethanation in terms of productivity and stability was attributed to the facilitated direct IET (DIET) between exoelectrogens and methanogens via magnetite particles. *Methanosaeta* was the predominant methanogen group in the experimental reactors and likely played a key role in both DIET-mediated carbon dioxide-reducing and aceticlastic methanogenesis.
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1. Introduction

Anaerobic digestion (AD) is an attractive option with which to treat organic wastes because AD stabilizes pollution loads and produces biogas (primarily comprising methane and carbon dioxide) simultaneously. AD is a multi-step biological process mediated by diverse microbial groups, and therefore its performance relies on the harmonized activity of different microbes involved in the system. Microbes in AD communities form syntrophic relations to overcome energy barriers and break down compounds more easily. Interspecies electron transfer (IET) between fatty acid-oxidizing bacteria and hydrogenotrophic methanogens with hydrogen as the primary electron carrier is an important syntrophic process that plays a pivotal role in stable methanogenesis (Stams et al., 2012). The syntrophic partners sense redox conditions and affect one another's metabolism. Fatty acid oxidation accompanied by hydrogen evolution is inhibited whereas hydrogenotrophic methanogenesis is facilitated at high hydrogen partial pressures, and vice versa. Therefore, a disruption of this syntrophy can lead to an excessive accumulation of volatile fatty acids (VFAs) that results in a process upset.

Recently, direct interspecies electron transfer (DIET) between exoelectrogenic bacteria and methanogens via conductive materials has been reported (Morita et al., 2011; Kato et al., 2012). DIET is energetically more advantageous than conventional IET because DIET does not require the multiple enzymatic steps to produce hydrogen to function as an electron shuttle (Lovley, 2011). Several recent studies have demonstrated that stimulating DIET by adding conductive material facilitated methanogenesis. Liu et al. (2012)

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reported that DIET was promoted by the presence of granular activated carbon (GAC) and attributed the enhanced methanogenesis to the high conductivity of GAC facilitating electronic connections between cells. Similar observations were made in recent studies with biochar, a GAC precursor using methanogenic co-cultures (Chen et al., 2014). Kato et al. (2012) reported a significant acceleration of methanogenesis by the supplementation of a microbial consortium enriched from soil with magnetite (Fe_3O_4), a conductive iron oxide. Those authors suggested that DIET using magnetite particles as conduits of electrons stimulated methanogenic activity. Another study reported that the methanogenesis rate from propionic acid was greatly enhanced by the stimulation of electric syntrophy between propionate-oxidizing bacteria and methanogens via magnetite particles (Cruz Viggi et al., 2014).

Previous findings suggest that DIET can provide an interesting possibility to improve the energy balance and economic feasibility of an anaerobic digester. The influence of stimulated DIET may be more complicated in mixed-culture systems with complex microbial communities, which would have greater chances of containing more microbes potentially involved in electric syntrophy than defined co-culture communities. Most previous studies have investigated DIET in methanogenic communities using synthetic media containing ethanol or organic acids under co-culture conditions. A recent study from the authors' group demonstrated the positive effect of conductive iron oxides, particularly magnetite, on the biomethanation of cheese whey, a waste product of cheese production, in batch mode (Baek et al., 2015). However, little has been learned regarding the long-term influence of magnetite supplementation in a continuous AD process treating real waste stream. To address this gap, the present study seeks to monitor and compare the performance of continuous anaerobic digesters with and without the addition of magnetite over a period of one year. For comprehensive insights into the underlying ecology, the physicochemical and microbial responses to the presence of magnetite were comparatively analyzed between the reactors during the operation. The primary focus of this study is whether magnetite supplementation facilitates methanogenesis and helps avoid process imbalance in continuous culture and whether its effect is maintained for a long period. The outcomes of this study can help improve our understanding of the complex syntrophic relations involved in AD and open new possibilities for high-rate biomethanation.

2. Materials and methods

2.1. Bioreactor operation

Two anaerobic continuously stirred tank reactors (CSTRs) with a working volume of 2 L (total volume, 2.4 L), namely RC and RM, were operated with cheese whey as substrate for 376 days. Each reactor was initially filled with equal volumes of whey (diluted to 5 g/L as soluble chemical oxygen demand [COD]) and anaerobic sludge from a biogas plant treating sewage sludge (seeding ratio, 50% [v/v]). The physicochemical characteristics of the seed sludge and the whey substrate are shown in Table 1. The soluble-to-total COD ratio of the whey substrate was as high as 94.7% whereas the volatile-to-total-solids ratio was 89.2%. This result indicates that the whey substrate is highly organic and that the majority of the organic matter involved is soluble. Whey was selected as the substrate because it contains the majority of the essential nutrients for microbial growth and has been widely treated anaerobically with no additives. The reactors were run in batch mode during the first 5 days for start-up and then switched to continuous mode with daily feeding. RM was supplemented with magnetite (particle size, 100–700 nm; Fig. S1) through the substrate to achieve a final Fe concentration of 20 mM; RC was not amended and served as a control. The experimental period was divided into six and four phases for RC (PC1 to 6) and RM (PM1 to 4), respectively, according to the operating conditions. The time period and operating conditions for each phase are presented in Table 2. Both reactors were maintained at approximately pH 7.0 and 35 ± 2 °C throughout the experiment with automatic pH and temperature control units. The reactors were continuously agitated by bottom stirring for complete mixing of the mixed liquor.

Table 1
Physicochemical characteristics of inoculum and substrate.

Parameter	Unit	Anaerobic sludge	Cheese whey
Total COD	mg/L	13,356 (84)[a]	5114 (9)
Soluble COD	mg/L	620 (47)	4845 (37)
Total solids	mg/L	17,000 (236)	5550 (71)
Total volatile solids	mg/L	10,167 (236)	4950 (71)
Total suspended solids	mg/L	14,500 (236)	250 (71)
Volatile suspended solids	mg/L	9333 (0)	100 (0)
C	%	–[b]	35.3 (0.3)
H	%	–	7.3 (0.1)
O	%	–	45.8 (0.5)
N	%	–	1.5 (0)
S	%	–	0.2 (0)

[a] Standard deviations are in parentheses.
[b] Not measured.

2.2. DNA extraction

Total DNA was extracted from the reactor samples of interest using an automated nucleic acid extractor (ExiProgen, Bioneer, Korea) according to the manufacturer's instructions. One milliliter of a sample was pelleted at 13,000*g* for 3 min, and the supernatant was decanted. The pelleted fraction was then washed with distilled water by repeated resuspending (up to 1 mL), decanting (900 μL), and pelleting (13,000*g* for 3 min) to remove impurities. A 200-μL portion of the final resuspension (in 1 mL) was loaded onto the extractor with the ExiProgen Bacteria Genomic DNA Kit (Bioneer, Korea). The purified DNA was eluted in 200 μL of elution buffer and stored at −20 °C until use.

2.3. Denaturing gradient gel electrophoresis

Denaturing gel gradient electrophoresis (DGGE) was employed to analyze the microbial community structure in the reactors. Archaeal and bacterial 16S rRNA gene fragments were prepared by polymerase chain reaction (PCR) using domain-specific ARC787F/1059R and BAC338F/805R primer sets, respectively (Yu et al., 2005). PCR amplification was conducted in a two-step thermal program: initial denaturation at 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C (Kim and Lee, 2014). The resulting PCR fragments (20 μL) were electrophoresed on 8% (w/v) polyacrylamide gels (denaturant gradient, 35–65% for archaea and 25–65% for bacteria) for 16 h at 80 V in a D-code system (Bio-Rad, USA). After electrophoresis, the DGGE gels were stained with SYBR Safe dye (Molecular Probe, USA) and scanned under blue light (470 nm) for documentation. Bands of interest were cut from the gels and eluted in 40 μL of sterile water. The recovered DNA fragments were reamplified, purified, cloned, and sequenced as previously described (Kim et al., 2013). The retrieved sequences were compared against the GenBank and RDP databases. The RDP Classifier was used for taxonomic classification at a confidence threshold of 80%. The nucleotide sequences obtained in this study have been deposited in the GenBank database under accession numbers KX255698-KX255716.

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