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Enhanced methanogenic degradation of cellulose-containing sewage via fungi-methanogens syntrophic association in an anaerobic membrane bioreactor

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ABSTRACT

An anaerobic membrane bioreactor was configured for methanogenic degradation of cellulose-containing sewage. The degradation performance and microbial changes were evaluated under five hydraulic retention times (HRTs). The results indicated the methane production was largely enhanced with 92.6% efficiency of chemical oxygen demand (COD) converting to methane and 80% proportion of methane in produced biogas, meanwhile the biomass yield presented the fewest at the shortest HRT 8 h. Enhanced methane production with decreased biomass yield was attributed to an association between fungi and methanogens. Microbial analysis showed fungi *Basidiomycota* and methanogen *Methanoregula* apparently established the association, especially *Basidiomycota* reaching 93% relative abundance at HRT 8 h. Specific methanogenic activity (SMA) and biochemical methane potential (BMP) tests suggested the association was derived from H_2 production by fungi and H_2 consumption by methanogens, during the process of cellulose degradation. The methanogenic degradation of cellulose-containing sewage was markedly promoted via the fungi-methanogens syntrophic association.

1. Introduction

The most abundant type of wastewater, sewage is a valuable resource containing water, nutrients and energy, and is worthy of recovery and reuse. The use of organic wastewater as a source of energy has a long history, with the anaerobic conversion of organic pollutants into methane gas dating back to the 18th century (Speece, 2008). However, through the conventional practice of anaerobic sewage treatment, only a portion of the energy potential is captured, mostly due to the slow growing nature of methanogenic organisms in the system. Besides, determining the required reactor volume in the case of treating low-strength wastewater like sewage requires deciding the appropriate hydraulic loading rate (HLR) rather than the organic loading rate (OLR) which has been widely consulted in the design of high-strength anaerobic reactors. A high HLR results in a short hydraulic retention time (HRT) i.e. less than 24 h, which may result in biomass washout if the biomass retention and liquid retention are coupled in a reactor. With the increased application of membrane technology to wastewater treatment, anaerobic bioprocesses are being incorporated with membrane separation in a membrane bioreactor to increase biomass concentration in the bioreactor (Chen et al., 2016a). In an anaerobic membrane bioreactor (AnMBR), high microorganism concentrations can be sustained under a reasonably high HLR and sufficient mixing due to the complete decoupling of the solid retention time (SRT) from HRT (Wang et al., 2012).

The diameters of the archaea, bacteria and fungi, the three domains related to microbial digestion, are larger than or comparable in size to the membrane pores used in microfiltration which range in diameter from 0.1 to 0.2 μm. The average diameters of the archaea and bacteria are 0.5 μm (Wakeham et al., 2003), and fungi are in the 0.1 to 10 μm range (Bauer et al., 2008), respectively. The microbial aggregates derived from the co-cultures and EPS flocculation are also larger, with a diameter ≥ 1 μm. The resultant solid-liquid separation by membrane enables the retention of microbe cells, allowing for their co-occurrence.

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The focus on previous studies has largely been on the interactions of archaea with bacteria, namely those of methanogens with fermentative bacteria and acetogens. It has been generally concluded that a balanced methanogenic degradation process in a anaerobic bioreactor occurs because of the co-culture of bacteria and methanogens, and their syntrophic association results in hydrolysis, acidogenesis, acetogenesis and methanogenesis in turn towards complex organic compounds (Stams and Plugge, 2009). However, it has also been reported that fungal populations can form a syntrophic interaction with methanogens for methanogenesis in anaerobic or anoxic environments. The association of fungi-methanogens is mainly associated with the natural environment, i.e. in the soil and the intestinal tracts of herbivorous animals, for the sake of cellulose fermentation (Marvinsikkema et al., 1990; Orpin and Joblin, 1997), and have rarely been observed in man-made anaerobic bioreactors.

Sewage commonly contains a certain amount of cellulose fibers which are mainly from used toilet paper. Cellulose, as a kind of carbohydrate, is rich with carbon sources, but its microbial hydrolysis rate is quite slow. Because it does not change during transport in the sewer network and is difficult to degrade in the biological treatment unit, most of the cellulose is removed by sieve or is precipitated in the primary treatment in the wastewater treatment plant. In a membrane bioreactor, particles of cellulose with diameters in the range of 1 to 1.2 mm on average (Ruiken et al., 2013) is retained in the mixed liquor by the membrane. Since fungi have been observed to grow on cellulose fibers in many studies (Baldrian and Valaskova, 2008), it is assumed that these fibers enter the membrane bioreactor with the sewage influent and fungi populations may inhabit in the system.

In this study, a submerged AnMBR was set up for the methanogenic degradation of cellulose-containing sewage. The AnMBR were operated under five HRTs and the microbial samples were collected when the reactor performance was stable at each HRT. High throughput sequencing was used to analyze the 16s rRNA gene of archaea and bacteria, and 18s rRNA gene of eukaryota, to investigate their community structure and population composition under different operating HRTs. The dynamic relation between microbial community descriptors and methane production behavior was then analyzed. Particular attention was given to fungi multiplication and their possible syntrophic association with methanogens.

2. Materials and methods

2.1. Reactor setup and operation

A completely mixed AnMBR with an effective volume of 6 L was operated to treat synthetic sewage. Solid-liquid separation was performed using a micro-filtration membrane module (Kubota Membrane Cartridge, Japan) with a total surface area of 0.116 m^2. Two peristaltic pumps (Model 7518-10, Cole-Parmer, USA) were individually used to feed the influent into the reactor and withdraw permeate from the membrane. The produced biogas was recycled by a diaphragm pump (APN- 085 LV-1, Iwaki, Japan) to scour the membrane surface for fouling control via an air diffuser. A digital pressure meter (AP-V85, Keyence, Japan) was installed between the membrane module and the permeate pump to record trans-membrane pressure (TMP). Biogas production was measured according to the volume of biogas collected in a wetted gas holder. The reactor was operated at 25 °C by means of a water bath. More schematic description of the AnMBR were detailed in a previous study (Chen et al., 2016b).

Anaerobic digested sludge from a full-scale sewage treatment plant (Sendai Southern Gamo Purge Center, Japan) was used as the seed sludge. The reactor was fed with synthetic sewage that consisted of cellulose (by toilet paper) of 150 ± 50 mg/L, chemical oxygen demand (COD) of 670 ± 100 mg/L, ammonia nitrogen (NH_4^+-N) of 40 ± 15 mg/L and orthophosphoric phosphorus (PO_4^{3-}-P) of 5 ± 2 mg/L, according to the chemical composition described elsewhere (Chen et al., 2017a). As shown in Table 1, five different HRTs (48, 24, 16, 12 and 8 h) were implemented in the reactor, resulting in average OLR of 0.3, 0.7, 1.0, 1.4 and 2.0 gCOD/L/d respectively.

Table 1
AnMBR operation parameters.

Duration (day)	1–29	30–67	68–91	92–120	121–150
HRT (hours)	48	24	16	12	8
OLR (gCOD/L/d)	0.3	0.7	1.0	1.4	2.0
Membrane flux (L/m^2 h)	1.08	2.17	3.25	4.29	6.46

2.2. Microbial population analysis

Mixed liquor samples were collected on Day 20, 62, 83, 115 and 142, when the AnMBR could be deemed to have achieved its steady-state operation, respectively at HRT 48, 24, 16, 12 and 8 h. DNA was extracted with the FastDNA Spin Kit for Soil (MP Biomedicals, CA) according to the manufacturer's instructions. The polymerase chain reaction (PCR) targeting 16s rRNA genes was performed using the forward primer B341F (CCTACGGGNGGCWGCAG) and the reverse primer B785R (GACTACHVGGGTATCTAATCC) for bacteria, and primers A349F (GYGCASCAGKCGMGAAW) and A806R (GGACTACVSGGGTATCTAAT) for archaea. The 18s rRNA gene PCR was performed using the primers EF4 (GGAAGGGRTGTATTTATTAG) and NS2 (GGCTGCTGGCACCAGACTTGC) for eukaryota. PCR amplification was performed with a LightCycler® 96 fluorogenic quantitative PCR thermal cycler (Roche, Switzerland) using a 25 μL mixture containing 2.5 μL of the DNA extraction solution, 12.5 μL KAPA Hifi HotStart ReadyMix (KAPA Biosystems), 0.25 μL of the forward primer (25 μM), 0.25 μL of the reverse primer (25 μM) and 9.5 μL of PCR-grade water. Thermal cycling was performed as follows: 95 °C for 3 min and 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. Finally a 5 min extension step at 72 °C was performed. The PCR-amplified DNA products were separated by electrophoresis in a 2% agarose gel (BioWest Agarose) at 120 V for 30 min. The products were purified using the QIAquick PCR purification kit (Qiagen). The DNA concentration was quantified with a Qubit 3.0 fluorometer (Life Technologies). All samples were extracted in duplicate.

The obtained sequence fragments were assembled using Flash software. The Mothur program was used to obtain high-quality sequences for subsequent analysis. The UCHIME was then used to remove chimera sequences, and sequences with > 97% similarity were clustered to form operation taxonomic units (OTUs). The Ribosomal Database Project (RDP) was used for alignment at a confidence threshold of 80% (Wang et al., 2007) and the SILVA database was chosen as reference in the process (Pruesse et al., 2007). Nonmetric multidimensional scaling (NMDS) was introduced to visualize the changes in the microbial community at various HRTs, in accordance with the process outlined by (Miura et al., 2007). In addition, a principal component analysis (PCA) was implemented to analyze the similarity of microbial consortia.

2.3. Batch tests

Specific methanogenic activity (SMA) tests were carried out in 120 mL serum bottles placed in a water bath at 25 ± 1 °C. For each bottle, 40 mL of mixed liquor taken from the reactor was inoculated and the total liquid volume was ultimately set at 80 mL by adding deionized water after adding necessary carbon sources. Acetate and H_2/CO_2 (80:20) as carbon sources were used for testing aceticlastic SMA (SMA-acetate) and hydrogenotrophic SMA (SMA-H_2), respectively. For the SMA-H_2 tests, 1.4 atmospheric pressure was maintained by injecting H_2/CO_2 to the headspace during the whole process. For the SMA-acetate tests, sodium acetate was added to realize an initial COD concentration of 2000 mg/L. The headspace of the bottles fed with H_2/CO_2