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Impact of temperatures on microbial community structures of sewage sludge biological hydrolysis

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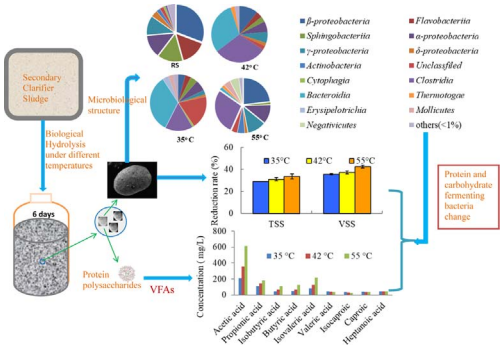
GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the biological hydrolysis performance at 35 °C (BH35), 42 °C (BH42), and 55 °C (BH55) and the effect of temperatures on microbial communities of the hydrolyzed sludge. The results showed that the suspended solid reduction, volatile fatty acids (VFA) production, and biogas production increased with the BH temperatures. VFAs produced in the sludge BH included acetic acid, propionic acid, isobutyric acid, butyric acid, and isovaleric acid with the fractions of acetic acid increased with BH temperatures. The Illumina MiSeq sequencing analysis showed that the microbial taxonomic structures of the BH systems varied with BH temperatures. It was found that *Acidaminobacter* at 35 °C, *Proteiniphilum* and *Lutispor* at 42 °C, and *Gelria* at 55 °C were the main protein fermenting bacteria genera, while the carbohydrate fermenting bacteria might belong to the genera of *Macellibacteroides* and *Paludibacter* at 35 °C, *Fronticella* at 42 °C, and *Tepidimicrobium* at 55 °C.

1. Introduction

Anaerobic digestion (AD) of activated sludge has gained increasing interests due to its essential role in reducing carbon footprints of wastewater treatment plants via energy recovery and waste reduction. Biological hydrolysis/acidification (BH) is a widely used pre-treatment process to increase the methanogenic degradation of complex organic compounds for methane production (Ding et al., 2017; Gao et al., 2011; Ucisik and Henze, 2008; Wang et al., 2014; Yang et al., 2015a). Temperature is one of the key BH process parameters that can exert a profound impact on the hydrolysis performance (Chu et al., 2008; Duarte et al., 2015; Liu et al., 2013). Although the effect of BH conditions on the biomass hydrolysis performance have been assessed by a number of studies (Chu et al., 2008; Zhang et al., 2009), knowledge of microbial communities structures in sludge BH systems is far from adequate for understanding sludge BH mechanisms.

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The anaerobic conversion of organics into methane gas involves the hydrolysis, acidogenesis, acetogenesis, and methanogenesis steps (Lee et al., 2014). Hydrolysis and acidogenesis bacteria, or the so-called primary fermenters, catalyze the extracellular hydrolytic degradation of polymers into oligo- or monomers and intracellular conversion of sugars, amino acids, fatty acids and other monomers into fatty acids, lactate, alcohols, etc. Acetogenesis bacteria, or the secondary fermenters, degrade products of the primary fermentation into acetate, H_2, and CO_2. The methanogenesis converts acetogenesis products into methane gas via two main pathways: acetoclastic and hydrogenotrophic CH_4 production (Da Silva et al., 2015). The complete conversion of complex organics to methane relies on syntrophic interactions of primary fermenters, secondary fermenters, and methanogens because mutual metabolic dependencies between different groups of anaerobic microorganisms eliminate the accumulation of intermediate hydrolysis products, making the conversion of complex organics to methane thermodynamically favorable (Schink and Stams, 2013). The syntrophic interactions in the sludge BH systems will depend on the microbial community structures and compositions. Thus, the determination of bacterial and archaeal community structures of BH systems would be of great importance for the understanding of microbial syntrophic interactions and process mechanisms of the sludge biological hydrolysis.

Illumina MiSeq Sequencing is an effective method for the characterization of microbial community structures. Compared to the conventional cultivation and biological molecular methods, Illumina MiSeq Sequencing can generate an enormous number of sequences, providing an excellent platform for the analysis of microorganism communities in wastewater treatment systems (Lin et al., 2016; Sheng et al., 2017; Xie et al., 2016; Zamanzadeh et al., 2016). With the use of Illumina MiSeq sequencing platform, Xie et al. (2016) revealed that the phyla of *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* were dominant in the hydrolysis acidification reactors treating dyeing wastewater. Lin et al. (2016), who investigated the effect of temperature on microbial communities in the anaerobic digestion of organic food waste, revealed that the phyla of *Firmicutes*, *Chloroflexi*, *Bacteoridetes*, and *Actinobacteria* were dominant under mesophilic conditions while the phyla of *Firmicutes*, *Thermotogo*, *Synergistales* dominated under thermophilic conditions.

The objective of this study was to investigate the effect of temperature on microorganism communities in the sludge BH systems. Temperature is one of the most critical process parameters for the sludge BH treatment. It can exert a critical impact on the types of microorganisms, fermentation pathways, and end-products of sludge BH. In this study bench-scale hydrolysis and biochemical methane potential (BMP) tests were carried out to assess the performance of sludge BH at 35 °C, 42 °C, and 55 °C. The high throughput Illumina MiSeq sequencing platform was used to determine the microbial community structures at the BH temperatures tested. Based on the MiSeq sequencing results, the characteristics of microbial community structures, syntrophic interactions and main functional fermenting bacteria under mesophilic and thermophilic BH conditions were discussed.

2. Material and methods

2.1. Sludge source and hydrolysis experiments

The raw sludge (RS) and seed sludge used in the BMP tests were taken, respectively, from the secondary clarifier and the anaerobic digester of Guelph Wastewater Treatment Plant, Ontario, Canada, and were transported to the laboratory within 2 h.

For the BH experiments, the RS was filled into a 1.5 L plastic bottle without addition of seed sludge, flushed with N_2 for 1 min, and then sealed for hydrolysis reactions. The hydrolysis experiments at 35 °C, 42 °C, and 55 °C were carried out in parallel by placing these sludge-filled plastic bottles into shaking incubators at the desired temperatures for six days. The sludge solubilisation caused by the 6-day hydrolysis was assessed by examining the change in the sludge TSS and VSS contents according to the Standard Methods (APHA-AWWA-WEF, 2005). The VFA compositions were measured using an Agilent 5890 gas chromatography (GC) (Agilent Technologies, US) with an Agilent J & W GC column (DB-FFAP 30 m × 0.25 mm) and a flame ionization detector (FID). The injector and detector temperatures were 250 °C and nitrogen was used as the carrier gas. The oven temperature was increased from 80 °C to 120 °C at a rate of 20 °C/min and held at 120 °C for 4 min, and then increased from 120 °C to 220 °C at a rate of 6.1 °C/min and held at 220 °C for 5 min. Volatile fatty acid mix standards (Sigma-Aldrich, Canada) including acetic, propionic, isobutyric, butyric, isovaleric, valeric, 4-methylvaleric (isocaproic), hexanoic (caproic), and heptanoic acids were used for the peak identification and standard curve determination.

2.2. Biochemical methane potential test

The biogas production from the hydrolyzed sludge was assessed using the BMP method. The BMP test procedure followed those of Owen et al.(1979) and Angelidaki et al. (2009) with modifications. Briefly, the main steps included (1) filling 30 mL of the hydrolyzed sludge and the equal volume of seed sludge into a 125 mL serum bottles (American Scientific Products, McGraw Park III); (2) flushing the headspace of the bottles with N_2 for 15 s; and (3) capping the BMP bottles with rubbers and aluminum crimp; and (4) placing the sealed BMP bottles upside down in a shaking incubator (New Brunswick Scientific C25) with the temperature setting at 35 °C. The BMP tests lasted for 10 days and the biogas produced during the BMP test periods was measured using glass syringes (Popper and Sons Inc.) fitted with a disposable needle. BMP bottles only filled with AD seed sludge and MilliQ water were used as the blanks to estimate the biogas generated from the seed sludge. Triplicated BMP bottles were used for each of the hydrolysis temperature conditions and the blanks.

2.3. DNA extraction

The PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc.) was used to extract DNA from the raw sludge and hydrolyzed sludge. The sludge samples were centrifuged at 10,000*g* for 10 min and the pellets were used for DNA extraction according to the PowerSoil kit manufacture's protocol. The extracted DNA was eluted using 100 μL sterile DNA-Free PCR Grade Water. The extracted DNA was quantified by using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Canada) and stored at −20 °C for further use.

2.4. 16S rDNA gene amplification and Illumina MiSeq sequencing

The primer pairs of 515F (5′-GTGCCAGCMGCCGCGG-3′) and 926R (5′-CCGTCAATTCMTTTGAGTTT-3′) that have been demonstrated to have the high coverages of almost all phyla in metagenomic analyses (Baker et al., 2003) were used to amplify the V4 and V5 regions of bacterial 16S rRNA genes of the extracted DNA. The primer pairs of 519F (5′-CAGCCGCCGCGGTAA-3′) and 915R (5′-GTGCTCCCCCGCCAATTCCT-3′) were used to amplify the 16S rRNA genes of methanogenic archaeal (Wei et al., 2015). The PCR reaction agent consisted of the 1 μL template, 1 μL of 10 mM dNTPs, 1 μL of 10 μM of primers, 1 U of Phusion DNA Polymerase, and 10 μL of 5 × reaction buffer (New England Biolabs, USA). The bacterial and archaeal PCR conditions were programmed as: denaturation at 94 °C for 2 min, followed by 25 cycles for bacteria or 30 cycles for archaea at 94 °C for 30 s, 56 °C for 30 s, extension at 72 °C for 30 s; with a final extension of 72 °C for 5 min.

The Illumina Nextera XT Index kit (Illumina Inc., San Diego. CA, USA) was used to attach 5′ MiSeq adapter and barcode to the amplicons for multiplexing following the manufacture's protocol. The amplicons were purified using the DNA gel extraction kit of AxyPrepDNA (Axygen, China) and quantified by using the FTC-3000^{TM} real-time PCR before

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