Journal of Environmental Management 223 (2018) 85–91

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

The relationship between volatile fatty acids accumulation and microbial community succession triggered by excess sludge alkaline fermentation

Xiangkun Li*, Gaige Liu, Shuli Liu, Kaili Ma, Lingwei Meng

School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China

ARTICLE INFO

Keywords:
Excess sludge
Volatile fatty acids
Anaerobic alkaline fermentation
Microbial community
Illumina Miseq sequencing

ABSTRACT

The volatile fatty acids (VFAs) accumulation pattern and microbial community succession were studied during excess sludge (ES) alkaline fermentation at pH of 10.0 with expanded granular sludge blanket reactor over 5 cyclers. Microbial community shifted conspicuously as ES suffered alkaline fermentation. Both VFAs and acid-producing bacteria increased rapidly during the first 8 days fermentation time, and they showed a quite positive correlation relationship. In addition, soluble chemical oxygen demand (SCOD) also dramatically increased during the first 8 days, which implied 8 day was the optimum sludge retention time (SRT) for ES alkaline fermentation and VFAs accumulation time. Illumina Miseq Sequencing analysis indicated that *Clostridium*, *Bacillus*, *Amphibacillus* and *Peptostreptococcaceae* were the dominant bacteria genus to produce VFAs. Acetic acid took about 84% in total VFAs because among the total acid-producing bacteria most bacteria could produce acetic acid.

1. Introduction

With the wide application of wastewater biological treatment process, ES problem has been outstanding all around the world (Xin et al., 2015; Duan et al., 2016; Su et al., 2016). Containing large amounts of organic/inorganic contaminants, heavy metal and pathogenic microorganisms, ES, if not properly treated, could cause environmental pollution and even human health issues (Carre et al., 2010; Jie et al., 2014; Yu et al., 2018). The cost of ES treatment and disposal accounts for approximately 60% of the wastewater treatment plants' operating costs (Ma et al., 2016; Wei et al., 2018). Thus, the problem of ES treatment and disposal has been critical and challenging.

Anaerobic digestion (AD) is a widely used process to treat ES, which can not only stabilize sewage sludge, but also realize sludge reduction (Appels et al., 2008; Li et al., 2014; Abelleira-Pereira et al., 2015). Appels et al. reported that volatile solids destruction could reach 65.5% after 30 days fermentation. AD is basically consisted of three steps, namely hydrolysis, acidogenesis and methanogenesis (Yu et al., 2018). VFAs are produced during the process of acidogenesis, and can be utilized as biodegradable carbon source for enhanced biological nutrients removal instead of additional supply of organic chemicals (e.g. methanol and acetate) to lower wastewater treatment plants' cost (Elefsiniotis et al., 2004; Li et al., 2014; Hao and Wang, 2015; Xin et al., 2018). However, hydrolysis is confirmed to be the rate limiting step, and VFAs produced during acidogenesis can be consumed by methanogens in the later menthanogenesis step (Yan et al., 2010; Peng et al., 2018). Hence, many experiments have been conducted to accelerate organic matters hydrolysis and control menthanogenesis, including ultrasonic treatment (Zhuo et al., 2012; Zielewicz, 2016), thermal treatment (Hao and Wang, 2015; Kumi et al., 2016), acid and alkaline treatment (Jie et al., 2014; Wang et al., 2017), ozone treatment (Yang et al., 2015; Fall et al., 2018), bio-surfactant treatment (Huang et al., 2015; Guan et al., 2017), combined treatment (Assawamongkholsiri et al., 2013; Wu et al., 2017; Ke et al., 2018) and so on. Among these methods, anaerobic alkaline fermentation is proved to be effective in VFAs accumulation and ES fermentation (Yuan et al., 2006; Wang et al., 2017).

Anaerobic alkaline fermentation (pH > 9) (Li et al., 2014) can shorten the time of hydrolysis and inhibit methanogens activity for VFAs accumulation. Bench-scale tests proved that pH 10.0 could gain the most VFAs than other pHs (Yuan et al., 2006; Chen et al., 2007; Wu et al., 2009; Jie et al., 2014). Yuan et al. reported that after 8 days treatment of ES, VFAs concentrations were as follows: pH 10.0 (250.39mgCOD/gVSS) > pH 9.0 (173.22) > pH 8.0 (131.38) > pH 5.0 (78.08) ≈ pH 7.0 (77.86) ≈ pH 11.0 (77.33) > pH 6.0 (50.17) > blanktest (58.58) > pH 4.0 (32.78). But there is no agreement on the SRT (Yuan et al., 2009), such as 10 days, 11 days, 12 days and so on. As we all know, microbial community is closely related to ES functionality and stability (Hidaka et al., 2018). Although some researches have already been done using alkaline anaerobic fermentation,

* Corresponding author.
E-mail address: xkli312@163.com (X. Li).

https://doi.org/10.1016/j.jenvman.2018.06.002
Received 29 March 2018; Received in revised form 8 May 2018; Accepted 1 June 2018

there are few studies about the close relationship between acetic producing microbial community succession with VFAs accumulation. Denaturing gradient gel electrophoresis study indicated that *Firmicutes* bacterium clone D85 (AB658278) was main responsible for ES anaerobic fermentation (Yuan et al., 2009; Jie et al., 2014). And a study using the same method at pH 10 manifested that *Clostridium*, *Bacillus* and *Gamma proteobacterium* were the main functional bacteria species for VFAs accumulation (Li et al., 2014). But the related acetic producing bacteria abundance changes with VFAs accumulation were not talked about.

In this work, ES alkaline fermentation at 35 °C was operated in expanded granular sludge blanket reactor at pH of 10. The specific objectives were to: (a) demonstrate the boost of alkaline condition on organics solubilization and VFAs accumulation and composition to find the optimal SRT, (b) illustrate the microbial community composition and changes during the fermentation period using Illumina Miseq Sequencing method, (c) figure out the close relationship between acid producing microbial community succession with VFAs accumulation, find the predominant microbes to produce VFAs.

2. Methods

2.1. Excess sludge properties

The ES was taken from a secondary sedimentation tank of municipal wastewater treatment plant in Harbin, China. The sludge was concentrated by settling at 4 °C for 24 h. The detailed corresponding traits were showed in Table 1.

2.2. Operation strategy

The expanded granular sludge blanket reactor (Fig. S1) with a working volume of 2.5 L was used as the ES anaerobic alkaline fermentor in this study. The ES was adjusted to pH 10 by adding appropriate dosage of 4 M sodium hydroxide or 4 M hydrochloric acid every 12 h. The startup period was divided into two stages (every stage was 8 days) to enrich acid producing bacteria. In the first stage (0–8 d), 2.5 L ES was added to the reactor by one time and TVFAs increased to 283.4 mgCOD/gVSS on the 8th day from the initial 21.5 mgCOD/gVSS. The second stage was from 8th to 16th, during which 1/3 ES remained from stage one and 2/3 ES form wastewater treatment plant was added to the reactor to a total volume of 2.5 L, and TVFAs reached up to 476.7 mgCOD/gVSS, almost the same concentration as stabilization stage (Zhang et al., 2009a,b). Steady period included 5 successive cycles (every cycle was 20 days): I(0–20 d), II(20–40 d), III(40–60 d), IV(60–80 d), V(80–100 d), and the operation strategy was exactly like the stage two of startup period (Stab.1).

2.3. Analytical methods

The ES samples taken from the reactor were centrifuged at 10,000 rpm for 10 min, and then filtered through 0.45 μm mixed cellulose ester membranes for measurement (Jie et al., 2014). Parameters TCOD, SCOD, SS, VSS, NH_4^+-N and PO_4^{3-}-P were determined according to the Standard Methods (APHA, 2005). The analysis of VFAs concentration and composition was using an Agilent 7890A Gas Chromatography (GC), with a flame ionization detector (FID) and HP-FFAP capillary column (inner diameter of 0.25 mm and length of 25 m). Nitrogen was provided as the carrier gas at 35 mL/min. The temperature of injector and detector were 250 and 300 °C, respectively. The oven of the GC began at 70 °C, then increased at a rate of 25 °C/min to 170 °C, and held at 170 °C for an additional 2 min. Sludge samples were collected every 24 h and all the samples were analyzed in triplicate.

Table 1
Characteristics of the ES used in the experiment.

Parameter	Raw ES value[a]
pH	6.8 ± 0.2
TCOD (total chemical oxygen demand, mg/L)	26,561 ± 476
SCOD (soluble chemical oxygen demand, mg/L)	3727 ± 16
SS (suspended solids, mg/L)	33,830 ± 357
VSS (volatile suspended solids, mg/L)	15,840 ± 113
NH_4^+-N (ammonia nitrogen, mg/L)	397.5 ± 13.1
PO_4^{3-}-P (soluble phosphorus, mg/L)	260.1 ± 11.0
TVFAs (total volatile fatty acids, mgCOD/gVSS)	21.5 ± 2.1

[a] Results are the averages and their deviations of triplicate measurements.

2.4. DNA extraction and PCR amplification

Biomass samples was collected from the reactor at different times: 60 d (cycle IV), 62 d (cycle IV), 68 d (cycle IV), 80 d (cycle IV), 80 d (cycle V), 82 d (cycle V), 88 d (cycle V), 100 d (cycle V), and in order to simplify the description in the part of Illumina Miseq Sequencing, they were marked G1, G2, G3, G4, G5, G6, G7, G8, respectively. The samples were immediately stored at −80 °C for the following experiments.

Total genomic DNA was extracted by using Powersoil® DNA Isolation Kit (MoBio, Carlsbad, CA). The concentration of DNA was determined by Nano Drop® Spectrophotometer ND-2000 (Thermo Fisher Scientific, USA).

PCR amplification was performed in triplicate for further clone sequencing. Bacterial primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′ -GGACTACHVGGGTWTCTAAT-3′) were used to amplify 16S rRNA genes (Chu et al., 2015). The 20 μL PCR amplification reaction volume contained 1 × EasyTaq buffer, 250 μM dNTPs, 0.2 μM of each primer, 1 U FastPfu polymerase (TransGen, China), 15–30 ng of template DNA. The PCR amplification was started with an initial denaturation step at 95 °C for 2 min, and followed by 27 cycles of denaturation (95 °C, 30 s), annealing (55 °C, 30 s) and extension (72 °C, 30 s), and then ended with an extension step of 72 °C for 5 min in a GeneAmp 9700® thermocycler (ABI, USA). The triplicate PCR products were detected by 2% (w/v) agarose gel electrophoresis and recovered using an AxyPrep DNA Gel Extraction Kit (AXYGEN, China) (Chu et al., 2015).

2.5. Sequencing and bioinformatic analysis

QuantiFluor™-ST Fluorometer (Promega, USA) was applied to quantify the purified amplicons. According to the equimolar ratios of amplicons from all samples, a composite sequencing library would be structured. And then, paired end sequencing of the resulting library would be sent to an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co, Ltd. (Shanghai, China). Eventually, the sequences numbers of every sample were given in tables with an average length at 435.95 bp. The sequences of the strains of each sample would be categorized by phylum, class, order, family, genus or specie such that the information of every sample from the reactor with increased fermentation time would be better understood. Operational taxonomic units (OTUs) at 97% sequence which was identified by utilizing UPARSE inset in Qiime (Caporaso et al., 2010) could cluster effective sequences.

3. Results and discussion

3.1. ES hydrolysis

SCOD was mainly consisted of hydrolyzates and VFAs, thus sludge hydrolysis could be presented as SCOD concentration variation (Hatziconstantinou et al., 1996; Chen et al., 2007). Fig. 1 showed the changes of SCOD concentration and SCOD/TCOD ratio during the five anaerobic alkaline fermentation cycles. It was obvious that SCOD concentration increased rapidly during the first 8 days of each cycle (especially the first 2 days), then the increasing trend tend to be moderate until the end of the cycle. SCOD concentration increased