Start up of the EGSB reactor under the condition of poor nutrition and Microbial community analysis by next generation sequencing

Liao Runhua[1, a], Jiang Yan[1, b], Wang Weipan[1, c], Du Jie[1,d], Li Yueming[1,e] and Fan Bin[1,f]

[1]Jingdezhen Ceramic Institute, Jingdezhen 333403, China

[a]maxlrh@126.com, [b]519663575@qq.com, [c]1306617315@qq.com, [d]sallydj@126.com, [e]lym6329@126.com, [f]519663575@qq.com

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Abstract: The objective of this study was to investigate the successful start–up of poor nutrition simulated wastewater with sodium acetate carbon source in an EGSB reactor, and EGSB reactor inoculated the flocculent sludge from the anoxic zone in the municipal sewage treatment plant. The results demonstrated the COD removal efficiency can also achieve more than 80%, even reaching above 95%, when the COD concentration of influent was improved from 200, 500 to 1000 mg/L in the continuous operation of 73 days. Furthermore, the microbial communities of seeding source, sludge samples collected on the 43th day (A1) and at the end of operation (A2) were investigated with the next generation sequences (NGS) tool in the EGSB reactor. Analysis results showed that the microbial diversity decreased. At the bacterial phylum level, there was an apparent increase in relative sequences of *Chloroflexi* and *Euryarchaeota* (33.01% and 0.63% in A1, and 44.03% and 7.14% in A2), which was 19.40% and 0.54% in the inoculum. At the genus level, except unclassified genus, *Methanosarcina*, *Clostridium* and *WCHB1*-05 were found to be the main members, whose sum occupied 16.13% and 18.41% of the total sequences in A1 and A2, respectively.

Introduction

The expanded granular sludge bed (EGSB) reactor is the third generation anaerobic bioreactor developed on the basis of the Up-flow Anaerobic Sludge Blanket (UASB) reactor [1]. Compared with UASB reactor, it increases the part of influent recycling, which causes that the rising velocity of the liquid in the reactor is much higher than that of UASB reactor, and that sewage and microbial strengthen the contact between them. Furthermore, it is because of the unique technical advantages that the EGSB reactor can be used for a variety of organic wastewater treatment, and has high processing efficiency [2-4]. So far, in the domestic and foreign literature, EGSB reactor sludge were mostly adopt cultivating granular sludge in UASB reactor, and mainly treated the rich nutrition high concentration wastewater. However, it was rarely reported that poor nutrition low concentration wastewater was treated in an EGSB reactor. In the present study, EGSB reactor was adopt the flocculent sludge from the anoxic zone in the municipal sewage treatment plant, and the start–up of poor nutrition simulated wastewater with sodium acetate carbon source in an EGSB reactor was investigated. At the same time, the microbial communities of seeding source, sludge samples collected on the 43th day (A1) and at the end of operation (A2) were investigated with the next generation sequences (NGS) tool in the EGSB reactor.

Material and methods

Bioreactor, inoculation and synthetic wastewater

The schematic diagram of the bench-scale EGSB reactor used in this study is shown in Fig. 1. The Plexiglas EGSB reactor was 60 mm in diameter and 1100 cm in height, giving a total volume of 3.58 L and a working volume of 2.31 L. A peristaltic pump was used to introduce influent at the column bottom of the reactor. A gas-washing device was used to collect the generated CH_4 and CO_2 gas at the column top. A

three-phase separator was installed at the reactor top to keep the biomass within the reactor. Excess sludge was discharged from the bottom of the EGSB reactor. The liquid up-flow velocity was controlled by inner recirculation. The EGSB reactor was operated under mesophilic conditions (35 ± 1°C) [5]and its temperature was maintained by a water bath.

The seed sludge was domesticated from the anoxic sludge (mixed liquor volatile suspended solids (MLVSS):27.178 g/L) of the Fuliangxian Urban Sewage Treatment Plant, Jingdezhen, China, which was filtered through 0.2 mm Tyler mesh to eliminate most grit. The reactor was started up with the influent COD concentration at about 200 mg/L, and liquid up-flow velocity at 2.5 m/hr, hydraulic retention time (HRT) (22 h), and sodium acetate as carbon source. During 73 days of operation, the influent COD concentration of the synthetic wastewater gradually increased from 200 to 500, and 1000 mg/L. Simulated oligotrophic wastewater was made of sodium acetate as the substrate and tap water as solvent configuration, not to add other nutritional elements.

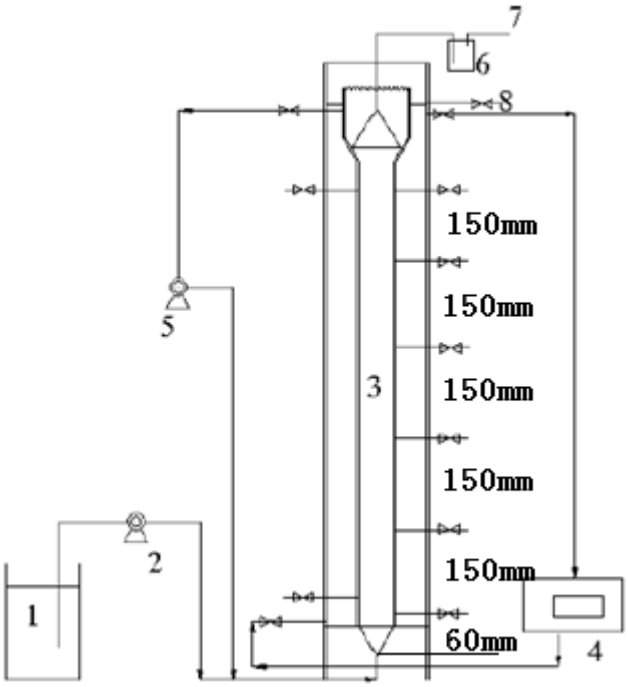

Fig. 1. Experimental apparatus and process flow chart of the EGSB reactor operated at 35±1℃, in which: (1) Influent tank; (2) Influent pump; (3) EGSB reactor (4) Water bath temperature control system;(5) Recycling pump; (6) gas sampler; (7) wet gas meter; (8) effluent water;

Chemical analysis

Wastewater parameters chemical oxygen demand (COD), Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) were measured according to the Standard Method[6]. A pH meter (pHS-25) was used to determine the pH values of liquid samples and Oxidation-Reduction Potential (ORP) were performed with the use of a MP551 conductivity meter (SANXIN Instrument and Meter Plant, Shanghai, China).

Microbial community analysis

The samples were subjected to total genomic DNA extraction using the Power Soil DNA extraction kit (Mo Bio, Carlsbad, CA) following the manufacture's procedure. Preparation of 16S rRNA gene amplicon and subsequent Illumina sequencing were conducted by the DNA Services Facility at the University of Illinoisat Chicago. Briefly, PCR amplification were conducted in triplicate reactions for all samples using the 515F/907R primer set that amplifies the V4-V5 region of the 16SrRNA gene[7-8]. Amplicons of the triplicate PCR reactions were pooled and then sequenced using an Illumina HiSeq 2000 instrument. De novo assemble of paired-end reads was performed with the software package CLC Genomics Workbench Version 6.0 (CLC bio, Cambridge, MA), and the resulting fastq files were trimmed using a cutoff quality score of Q15 and read length of larger than 200bp[9]. Only the forward reads were used for downstream analysis, as it was reported that including the reverse reads add little additional information[10].

After sequencing, the raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17). As the number of spurious phylotypes increases with sequencing effort, equal number of sequence

reads should be used to compare microbial community among samples to minimize the sequencing artifact[11]. In this work,40000 reads from every sample were randomly picked and grouped into operational units (OTUs) with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/). Chimeric sequences were identified and removed using UCHIME. Community diversity index (Chao1, PD, Simpson, Shannon diversity index) and rarefaction curves were generated using the MOTHUR program. The taxonomic assignment of OTUs was performed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database at 70% threshold[12].

Results and discussion

Start-up performance

The change of ORP, pH value and COD removal efficiency was shown in Figure 2 during the start-up process of the EGSB reactor. As can be seen from the figure 2, the COD removal efficiency reached more than 80% after the continuous operation of 15 days, which indicated that it was good adaptability for the poor nutrition simulated wastewater treatment in an EGSB reactor. In the meantime, the COD removal efficiency can also achieve more than 80%, even reaching above 95%, when the COD concentration of influent was improved from 200, 500 to 1000 mg/L in the continuous operation of 73 days. At this time, it was successful that the EGSB reactor started up. In the start-up process of the EGSB reactor, the pH value of influent, in the reactor and effluent ranged between 7.2 to 8.4, and the Oxidation Reduction Potential of effluent and in the reactor was between 0~-250 mv. The change trend of COD, pH and ORP was similar results with the previously reported literature [13-14].

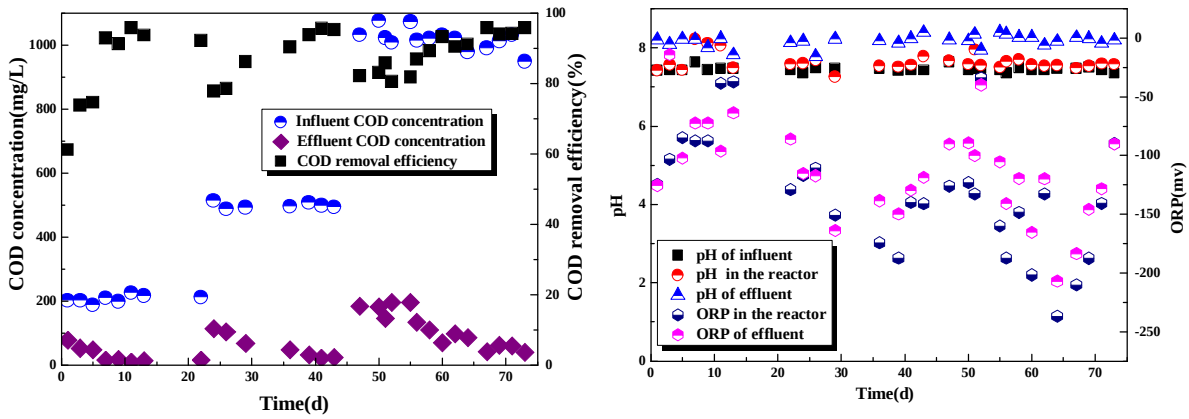

Fig. 2. Removal efficiency of COD, pH and ORP during the start-up and operation of an EGSB reactor

Microbial community

To reveal the shift in the microbial community structure during the start-up process of the EGSB reactor, the inoculum, and sludge samples collected on the 43th day (A1) and at the end of operation (A2) were used to pyrosequence the former region of the 16S rRNA gene using a 454 GS-FLX sequencer. The sampling was prepared as a mixture of 10 mL mixed liquor at three ports (60 mm, 210mm, and 360 mm from the bottom). A total of 220497 16S rRNA sequence reads were generated by the pyrosequencing of duplicate samples. After filtering the low quality reads using the RDP Initial Process in Pyrosequencing Pipeline (PP) and trimming the adapters, barcodes and primers, denoising, filtering out chimeras, the library size of the sample was normalized to 133114 high quality sequence reads and 4581 operational taxonomic units(OTUs) with similarity cutoffs of 3% were obtained from a single lane of an eight-lane pico-titer plate on the Genome Sequencer FLX Titanium system.

The sequencing depths were adequate to capture majority of the microbial diversity, as indicated by the rare faction curves approaching the asymptotic state. Microbial communities at the different stages of the

anaerobic digestion processes all showed very diverse microbial communities (Table 1). The Shannon index (H') for three samples ranged from 7.8 to 8.4, and showed a downward trend. And that variational trend of Simpson diversity index was the same as Shannon index from the Table 1. While variational trend of OTUs, Chao1, and PD increased first and then decreased, it indicated that the microbial diversity decreased with the increase of the influent load, after the stable operation of the EGSB reactor.

Table.1. Raw, effective bacterial reads, and plus numbers of OTUs, Chao1, PD, Shannon and Simpson of three activated sludge samples at level of 3% cutoff. (A1 = samples were taken after 43rd days of an EGSB operation, and A2 = samples were taken at the end of an EGSB operation).

Sample	Raw reads	Effective reads	OTUs	chao1	PD	shannon	simpson
Inoculum	72707	41635	1546	1770	108	8.4	0.99
A1	72239	42933	1595	1832	111	8.3	0.99
A2	75551	48546	1440	1763	103	7.8	0.98

Note: OTUs: operational taxonomic units; Shannon: Shannon's diversity index;
Chao-community richness. A higher number represents more richness;
Shannon - community diversity. A higher number represents more diversity;
The biodiversity indices (OTUs, Chao1, PD, Shannon and Simpson) were calculated based on normalization of the number of the filtered sequences from each sludge sample to 40000.

Fig. 3 showed the changes in diversity of key bacteria at start-up (inoculum), at the 43rd operation (A1), and at the end of operation (A2). Clear differences in the relative abundances were observed as EGSB reactor operation continued. At the bacterial phylum level, there was an apparent increase in relative sequences of *Chloroflexi* and *Euryarchaeota* (33.01%, 0.63% in A1 and 44.03%, 7.14% in A2), which was 19.40% and 0.54% in the inoculum. This increase was in accordance with the previous work of Chen et al.[15]. Meanwhile, the vast majority of the total sequence detected in inoculum such as *Proteobacteria* (23.30%), *Actinobacteria* (8.68%), *Acidobacteria* (4.86%), *Bacteroidetes* (10.80%), *Gemmatimonadetes* (1.24%) and *TM7* (1.32%) decreased in A1 and A2.

At the genus level, except unclassified genus, *Methanosarcina*, *Clostridium* and *WCHB1-05* were found to be the main members, whose sum occupied 16.13% and 18.41% of the total sequences in A1 and A2, respectively. In particular, there was a significant increase in *Methanosarcina* and *WCHB1-05* (6.46% and 5.87% in A2), which was only 0.05% and 1.03% in the inoculum. Meanwhile, the relative abundances of other genera such as *Hyphomicrobium*, *Methanobacterium*, *Methylosinus*, *Caldilinea*, *Turicibacter*, *Rhodoplanes*, *Nannocystis*, *Proteiniclasticum*, *Gemmata*, *Novosphingobium*, *Crenothrix*, and *Nitrospira* decreased in A1 andA2. EGSB reactor under the condition of poor nutrition is successfully start up, and the inoculation sludge was flocculent sludge from anoxic area instead of granular sludge. It indicated the EGSB reactor had a good adaptability, and the diversity of microbial community was also in line with the actual situation.

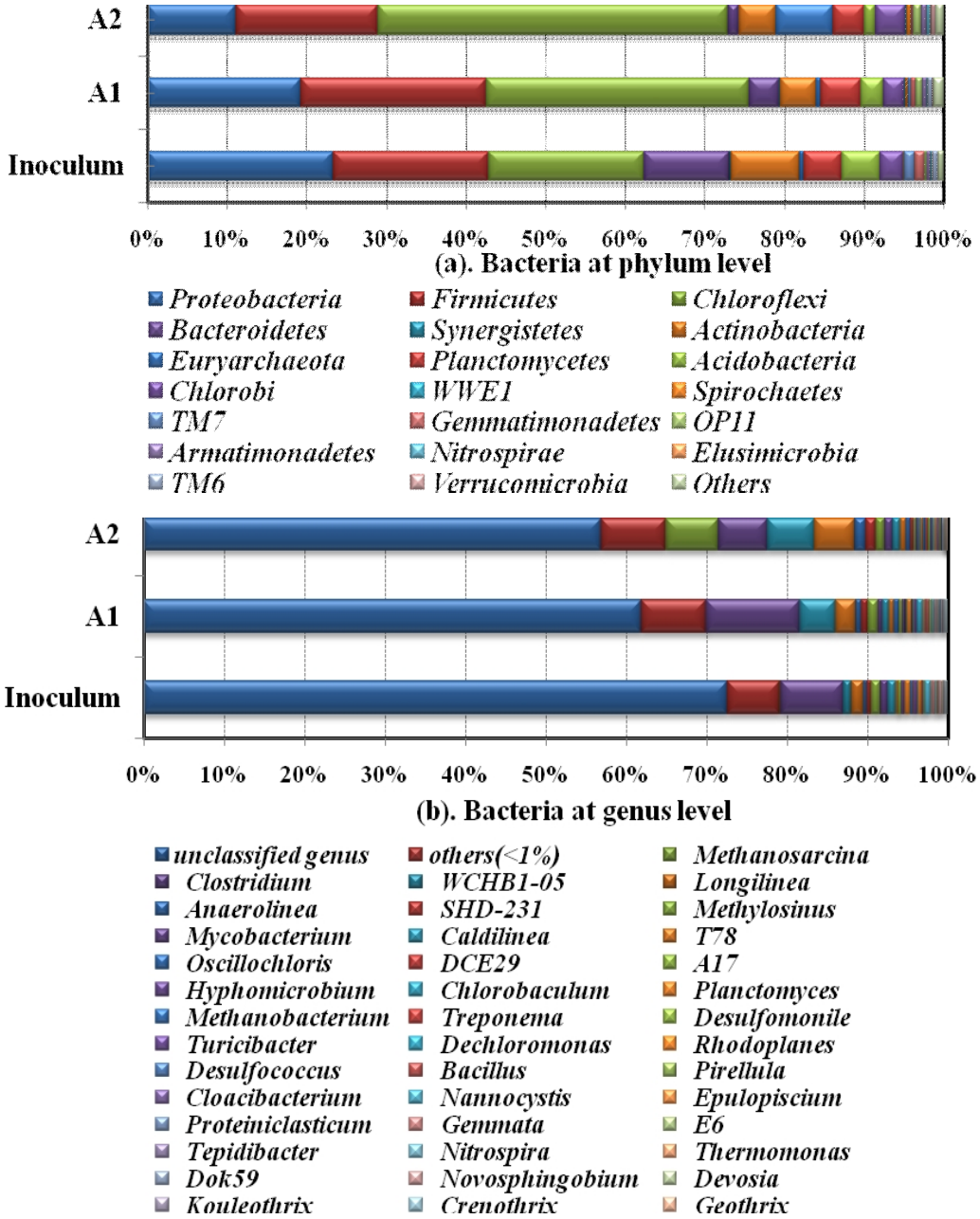

Fig. 3. Changes in diversity of key microbiology community by next generation sequencing (A1 = samples were taken after 43rd days of an EGSB operation, and A2 = samples were taken at the end of an EGSB operation).

Conclusions

poor nutrition simulated wastewater with sodium acetate carbon source in an EGSB reactor successfully start up, and EGSB reactor inoculated the flocculent sludge from the anoxic zone in the municipal sewage treatment plant. The results demonstrated the COD removal efficiency can achieve more than 90%, when the COD concentration of influent was improved 1000 mg/L in the continuous operation of 73 days. The NGS results showed there was an apparent increase in relative sequences of *Chloroflexi* and *Euryarchaeota* (33.01%, 0.63% in A1 and 44.03%, 7.14% in A2), which was 19.40% and 0.54% in the inoculum at the bacterial phylum level. there was a significant increase in *Methanosarcina* and

WCHB1-05 (6.46% and 5.87% in A2), which was only 0.05% and 1.03% in the inoculum at the genus level.

Acknowledgements

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