STUDY ON THE AEROBIC MICROBE POPULATION DYNAMICS OF BULKING SLUDGE IN WASTEWATER TREATMENT

WANG, S.^{1,2} – XIAO, X. L.^{1*} – CHENG, S. G.^{1,2} – LAI, F.¹ – QIAO, X. R.³

¹*School of Environmental Science and Engineering, Shaanxi University of Science and Technology, Xi'an 710021, China*

²*National Demonstration Center for Experimental Light Chemistry Engineering Education, Shaanxi University of Science & Technology, Xi'an 710021, China*

³*School of Arts and Sciences, Shaanxi University of Science and Technology, Xi'an 710021, China*

**Corresponding author*

e-mail: xxl0108@163.com, phone: + 86-136-3680-8617

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Abstract. Activated sludge process is one of the most common methods in wastewater treatment, however, bulking sludge is nerve-racking during the actual treatment. In this study, Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was employed to study the diversity of the micro-organisms of the untreated sludge and bulking sludge in the biological wastewater treatment. Simultaneously, microbe species and dominant microflora of the untreated sludge and the bulking sludge were analyzed. This paper provides a reliable theoretical basis to the prevention and control of the bulking of the activated sludge. The results indicate that the bulking of the activated sludge is mainly caused by the change of microbe population.

Keywords: *PCR-DGGE, the diversity of microbe, bulking sludge, filamentous, dominant microflora*

Introduction

The activated sludge treatment is an aerobic biological treatment. Under the aerobic condition, the organic matter in the wastewater is decomposed into inorganic materials, so as to purify the wastewater (Shao et al., 2005). Sludge bulking is a frequent and serious problem in plants using activated sludge, and the causes are fairly complex. Many researchers have studied this issue. The over-growth of many filamentous bacteria may cause the bulking problem (Henze et al., 2008). Simultaneously, theoretical studies on the overgrowth of the filamentous bacteria have also progressed (Eikelboom et al., 1975; Sezgin et al., 1980). The causes of sludge bulking can be divided into two types: the first is caused by the rapid multiplication of the filamentous bacteria in the activated sludge; and the other is caused by a large accumulation of highly viscous substances produced by bacterial colloid (Qin et al., 1989; Ma et al., 2012). In recent years, the metabolic diversity and genetic adaptability of microbes in sludge have been studied. Denaturing gradient gel electrophoresis (DGGE) fingerprint results indicated a shift in bacterial community composition during the granulation of aerobic granules, which was confirmed by 16SrRNA gene sequencing (Aqeel et al., 2015). Real-time quantitative polymerase chain reaction (qPCR) was used to study the abundance and community structure of ammonia-oxidizing bacteria in the activated sludge taken from different geographic

regions in China (He et al., 2018). The abundance of filamentous morphotypes and floc-formers able to store biopolymers were analysed by PCR-DGGE and 16S amplicon sequencing (Meunier et al., 2016). With the development of modern molecular biotechnology such as DNA extraction, it is possible to analyze the diversity of microbial species and heredity (Li et al., 2015; Jiang et al., 2014).

In this study, PCR-DGGE was employed to study the micro-biodiversity of the untreated sludge and the bulking sludge. The species and dominant microflora of microbes in the two kinds of sludge were compared, and the causes of sludge bulking were found, which provides a reliable perspective for solving the problem.

Materials and methods

Materials

The experiment material was collected from the acclimated sludge and filamentous bulking sludge in a wastewater treatment plant Xi'an, China.

Methods

PCR-DGGE include preparation of sample DNA, PCR amplification and denaturing gradient gel electrophoresis (Sun et al., 2009).

(1) Preparation of sample DNA

Bacteria acquired through Centrifuge collection were dissolved in 5mL extraction buffer (100 mM Tris·Cl, 100 mM EDTA-Na₂, 200 mM NaCl, 2% CTAB, pH 8.0) and were shaken at 37 °C for 30min. 0.75 mL 20% SDS was added in the solution above then water bathed it at 65 °C for 1 h. The supernatant was collected by centrifugation at 12000 rpm for 10 min. The volume (the same as the supernatant) of the mixture of phenol, Chloroform and Isoamyl alcohol, the ratio of which are 25:24:1, was extracted twice, 0.3 M of NaAC (pH 5.2) and ethyl alcohol which was twice as the volume as the extracted solution were added, and precipitated for 1 h at ambient temperature. After centrifugation (12000 rpm, 20 min) at 4 °C, the precipitate was collected and rinsed twice with 70% ethanol, then dried and dissolved in 50 µl TE.

(2) PCR amplification

PCR amplification primers used are F357(5'-CGCCCGCCGCGCCCGCGCCCGGCCCGCCCGCCCCCTACGGGAGG CAGCAG -3') and R518(5'- ATT ACC GCGGCTGCT GG -3') from bacteria 16SrDNA V3 Highly variable region for the analysis of the diversity of the bacteria in the activated sludge. PCR amplification primers used fungus NS1(GTAGTCATATGCTTGTCTC) and Fung-GC(GC-ATTCCCCGTTACCCGTTG) for the analysis of the fungal diversity.

(3) Denaturing gradient gel electrophoresis (DGGE)

D-Code Universal Mutation Detection System (Bio-Rad) was used for the DGGE of the PCR products.

Results and discussion

Analysis of the bacteria diversity of the untreated activated and bulking sludge

After DGGE analysis and sequencing, the gene sequence was compared with the NCBI database, and the results of the main bacteria identification in the untreated sludge and bulking sludge were shown in *Tables 1,2* and *Figures 1,2*.

Table 1. Identified bacteria species in the untreated sludge

Stripe number	Clone number	V3 region	Bacteria species generic name
1	> 8.15XDG123-1-M13-	194bp	<i>Zoogloea</i> sp.
2	> 8.20XDG123-3-M13-	194bp	<i>Sterolibacterium</i> sp.
3	> 8.15XDG123-4-M13-	194bp	<i>Uncultured bacterium</i>
4	> 8.15XDG123-5-M13-	194bp	<i>Pseudomonas</i> sp.
5	> 8.15XDG123-8-M13-	194bp	<i>Legionella pneumophila</i>
6	> 8.15XDG123-9-M13-	193bp	<i>Uncultured bacterium</i>
7	> 8.15XDG123-10-M13-	194bp	<i>Uncultured Aquabacterium</i> sp.
8	> 8.15XDG123-12-M13-	187bp	<i>Uncultured bacterium</i>
9	> 8.15XDG123-15-M13-	174bp	<i>Agrococcus lahaulensis</i>
10	> 8.15XDG123-18-M13-	194bp	<i>Uncultured Alcaligenes</i> sp.

Figure 1. Phylogenetic tree of the bacteria in the untreated sludge

Most bacteria in the untreated sludge belong to proteobacteria (including α -proteobacteria, β -proteobacteria and γ -proteobacteria) (Table 1) and showed a close genetic relationship. Sequence 2, 1, 4, 5, 10, 7 stand for the dominant microfloras in the activated sludge. Moreover, the bacteria represented by sequence 3, 8, 6 were not artificially cultivated and classified, and they also showed a close genetic relationship. The microbe community of the activated sludge is formed together with the same 9 microbes (Wang et al., 2015).

Significant reduction in the total number of the dominant bacteria in the bulking sludge is showed from 10 species in the untreated sludge to 6 species (Table 2) after the treatment and the microbial community changed greatly.

The strains represented by sequence 2~5 (Fig. 2) are in accordance with the four strains represented by sequence 5, 6, 8, 10 in the untreated sludge. Two strains of bacteria represented by sequence 1 and sequence 6 are new in the bulking sludge. Strains represented by sequence 1 and sequence 6 highly similar to the *Anaerolineaceae* and *Collinsella aerofaciens*, respectively.

Figure 2. Phylogenetic tree of the bacteria in the bulking sludge

Table 2. Identified bacteria species in the bulking sludge

Stripe number	Clone number	V3 region	Bacteria species
1	> 7.11XD1512-2(DG50-X-4)M13-	169bp	<i>Anaerolineaceae</i>
2	> 7.11XD1513-1(DG50-X-5)M13-	194bp	<i>Legionella pneumophila</i>
3	> 7.18XD1514-2(DG50-X-6)M13-	193bp	<i>Uncultured bacterium</i>

4	> 7.11XD1515-1(DG50-X-7)M13-	187bp	<i>Uncultured bacterium</i>
5	> 7.11XD1517-2(DG50-X-9)M13-	174bp	<i>Agrococcus lahaulensis</i>
6	> 7.11XD1518-3(DG50-X-10)M13-	170bp	<i>Collinsella aerofaciens</i>

Analysis of fungus diversity of the bulking sludge

After DGGE analysis and sequencing, the gene sequences were compared with the sequences in the NCBI database, and the main fungi in the bulking sludge were shown in Table 3.

Table 3. Identified fungi in the bulking sludge

Stripe number	Clone number	V3 region	Fungus species
1	> 7.24XD1534-6(DG50-Z-2)M13-	349bp	<i>Uncultured eukaryote</i>
2	> 7.24XD1535-1(DG50-Z-3)M13-	349bp	<i>Saccharomyces cerevisiae</i>
3	> 8.16XD1631-2(DG50-Z-4)M13-	347bp	<i>Unculture alveolate</i>
4	> 8.16XD1632-1(DG50-Z-5)M13-	346bp	<i>Hanseniaspora guilliermondii</i>
5	> 8.16XD1633-1(DG50-Z-8)M13-	337bp	<i>Uncultured eukaryote</i>

The bulking sludge contains five kinds of fungi (Table 3), besides bacteria, they constitute the microbial community of the sludge, and are a part of the dominant microflora in the bulking sludge. The genus can be identified by cloning V3 conservative regions. The result is shown in Figure 3.

Figure 3. Phylogenetic tree of the fungi in the bulking sludge

From Figure 3, the fungi represented by sequence 1, 3 and 5 are not artificially cultivated strains. The two fungus strains represented by sequence 2 and sequence 4 were closely related to *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* in the evolutionary relations. So the two fungi were identified as *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii*.

Analysis of the root causes of sludge bulking

(1) Changes of bacterial composition of non-treated and bulking sludge

From the results of DGGE analysis in *Figure 4*, it can be seen that bacterial composition changed greatly after sludge bulking, showing a decrease of bacterial species and the change of the amount of the dominant microflora. The microflora represented by sequence1 in the untreated sludge are *Zoogloea sp.* Under natural conditions, the *Zoogloea sp.* produces capsule that not only directly affects the growth and reproduction of the bacteria but also the living environment of the bacteria. Bacteria can improve the poor living environment with its capsule property, and prevent the phagocytosis and utilization by some bad cell tissue. And on the surface of some specific bacteria cells, it showed a strong specificified of attack and defense capability (Wang, 2016). Microfloras which are commonly used microbes in sewage treatment cluster in the common bacteria colloid, so that the sludge has a good gel-flocculation structure and sedimentation property (Zhong et al., 2010). The disappearance of *Zoogloea sp.* in the sludge is the direct cause of sludge bulking.

Figure 4. Bacteria domains in the untreated sludge and bulking sludge

(2) Changes of funguses composition of the untreated and bulking sludge

As can be seen from the DGGE bands in *Figure 5*, the fungus bands in the untreated sludge are few and unclear, indicating that the types and amounts of the funguses contained in the untreated sludge are small but the bulking sludge contains five kinds of fungi whose species and quantity are much more than the untreated sludge. Thus, the multiplication of fungi has changed the nutritional composition of the environment, absorbing a large number of nutrients, directly affect the growth of the bacteria such as *Zoogloea sp.* Thereby, the change of the composition of the microbe microfloras in the sludge, as well as the destruction of the flocs structure of the sludge lead to the sludge bulking.

Figure 5. Fungus domains in the untreated sludge and bulking sludge

Conclusion

From the analysis of the diverse microbes of untreated sludge and bulking sludge, it is seen that sludge bulking is mainly caused by the change of microbe microfloras.

(1) The sludge bulks because the microfloras in it changed greatly, the main signs are the decrease of the bacterial species and the dominant flora changes. Under natural conditions, *Zoogloea sp.* in the untreated sludge flora has the property of producing capsule and the bacteria which are the common microbes in wastewater treatment cluster in the common bacteria colloid, so that the sludge has a good gel-flocculation structure and sedimentation property. The disappearance of *Zoogloea sp.* is one of the direct causes of sludge bulking.

(2) DGGE analysis of the untreated sludge showed that only a very small amount of fungi were present in the activated sludge, which indicates that the activated sludge mainly consisted of bacteria, but five kinds of filamentous fungi (most of which have hyphae) in the bulking sludge were detected with the same method. The mass growth of these filamentous fungi has replaced the predominant bacteria such as *Zoogloea sp.* in the activated sludge, therefore, the destruction of the gel-flocculation structure of the sludge eventually leads to the filamentous fungus sludge bulking.

In the future studies, the changes of carbon source, nitrogen source and inorganic salts in sludge before and after bulking should be systematically analyzed. Combined with the study of microfloras changes and strain metabolism, the mechanism of change would be deeply revealed, which would lay a theoretical foundation for controlling the sludge bulking.

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