Structure Analysis of Garlic Wastewater Activated Sludge Bacterial Flora

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ABSTRACT. In order to explore the bacterial diversity and bacterial community structure of garlic wastewater activated sludge in SBR reactor, illumina-Miseq high-throughput sequencing technology was used to analyze the bacterial community structure and diversity of garlic wastewater. The results showed that the abundance of bacteria was higher on the whole, but the diversity of bacteria was different among different samples. Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria are the dominant bacteria phyla, all of which have the function of denitrification and dephosphorization to degrade organic matter, with abundance between 6.3% and 43.72%. The five dominant genera of Macellibacteroides, Petrimonas, Rhodobacter, Fluviicola and Saccharibacteria_generaincertae_sedis in activated sludge have the functions of degrading organic matter and denitrifying, with abundances ranging from 1.81% to 21.48%. The research results can provide reference for the treatment effect of strengthening SBR reactor.

1. Introduction
China's garlic planting area is about 700,000 hectares, accounting for more than 60% of the global garlic planting area, and garlic production accounts for more than 70% of the world's total production. According to incomplete statistics, the production of 1t garlic products will produce 30-40t garlic wastewater. Due to the complex composition of garlic wastewater, high concentrations of pollutants such as allicin, NH3-N, COD, BOD and TP, and allicin has a strong antibacterial effect, making the wastewater a high-concentration organic wastewater that is difficult to biodegrade. If directly discharged, it will cause serious harm to the environment [1]. At present, some research results have been achieved in the biological treatment of garlic wastewater, but there are few studies on the microbial community structure that plays an important role, which largely restricts the development of garlic wastewater biological treatment [2]. In this paper, 16Sr RNA gene Illumina-MiSeq high-throughput sequencing technology was used to analyze the microbial flora structure and diversity in the garlic wastewater activated sludge in the SBR reactor, and provide reference and advice for improving the efficiency of biological treatment of garlic wastewater.

2. Materials and Methods

2.1. Test equipment and test water
In this experiment, artificially simulated water distribution was used. Fresh garlic on the local market was used as raw material, peeled and squeezed, and the squeezed garlic juice was diluted to the required...
concentration with tap water. The water quality indicators of the simulated wastewater are shown in Table 1.

### Table 1 Test water quality index

| Sample  | COD (mg/L) | COD removal rate/% | NH3-N (mg/L) | NH3-N removal rate/% | TP (mg/L) | TP removal rate/% | pH               |
|---------|------------|--------------------|--------------|----------------------|-----------|------------------|------------------|
|          | inlet      | outlet             | inlet        | outlet                | Inlet     | outlet           |                  |
| 1        | 5183-5848  | 236-540            | 90-96        | 59-78                | 94-96     | 9-10.14         | 0.4-0.6          | 93-96.5          | 7±0.5            |
| 2        | 5183-5848  | 152-319            | 94-97.5      | 59-78                | 95-97     | 9-10.14         | 0.2-0.3          | 96-97            | 7±0.5            |

2.2. Collection of sludge samples

Using a sterile container, collect an activated sludge sample from each SBR reactor, numbered sample 1 and sample 2 respectively. At the same time, biofilm was collected from the wall of No. 2 reactor, numbered sample 3.

2.3. Extraction of total DNA from activated sludge and PCR amplification

The total DNA of activated sludge samples was extracted by OMEGA Kit E.Z.N.A.TM Mag-Bind Soil DNA Kit. Amplify its V3-V4 region gene fragment. Amplification primer: 341F CCTACGGGNGGCWGCAG and 805R GACTACHVGGGTATCTAATCC, PCR reaction system is 30μl. It is divided into two amplifications, and the first round of amplification reaction conditions are as follows: 94℃ 3min; 94℃ 30s, 45℃ 20s, 65℃ 30s, 5 cycles; 94℃ 20s, 55℃ 20s, 72℃ 30s, 20 cycles; 72℃ 5 min, 10℃. The second round of amplification conditions: 95℃ 3min; 94℃ 20s, 55℃ 20 s, 72℃ 30s, 5 cycles; 72℃ 5 min, 10℃. Each sample was directly sequenced after PCR amplification.

2.4. High-throughput gene sequencing data processing

Store Miseq sequence data in fq file format, use Prinseq software for quality control, remove bases with a quality value of less than 20, and use Usearch to remove chimera and sequences outside the target region. Under the condition of similarity of 97%, OTU clustering was carried out, sparse curves were constructed by using Mothur software, and the Chao1 Shannon and Coverage indexes of the samples were calculated.

3. Results and analysis

3.1. Bacterial diversity analysis

Through the statistical analysis of the sequencing results of the V3-V4 region, it can be seen from Table 2 that the effective sequences of the three samples are 85656, 88314, and 83344 respectively. The sequencing depth of the three samples reached 98%. In theory, the sequencing data has covered all the sequences in the sample. OTU clustering was performed on the sequences at 97% similarity level. The total number of OTUs obtained was 5416, and the number of OTUs obtained from 3 samples were 2142, 2245, and 2285 respectively. The Chao1 index is used to describe the richness of the community in the sample. The higher the Chao1 value, the higher the richness of the community species in the sample. At 97% similarity level, it can be seen from Table 2 that the Chao1 index of sample 2 is the largest, indicating that sample 2 has the highest abundance, sample 3 comes next, and sample 1 is the lowest. The Shannon index is used to indicate the microbial diversity index in the sample. The larger the Shannon value, the higher the community diversity. The Shannon index of sample 3 is relatively highest, indicating that the species diversity of sample 3 is the highest, sample 2 is the second, and sample 1 is the lowest. Randomly sample the sequences, count the number of sequences and OTUs of the sample samples, and draw a dilution curve. From the OTU dilution curve in Figure 1, it can be seen that the dilution curves of the three samples are flat, but still not saturated, indicating that the three samples have obtained most of the sample information, which can basically reflect the microbial community composition in the garlic wastewater sludge. The Shannon exponential dilution curve has reached a plateau, indicating that a larger sequencing volume will not cause a significant increase in species
diversity, and the analysis results based on the existing data volume are accurate and reliable [3].

| Sample ID | Seq num | OUT num | Shannon | Chao1    | Coverage |
|-----------|---------|---------|---------|----------|----------|
| 1         | 85656   | 2142    | 4.03    | 6737.50  | 0.98     |
| 2         | 88314   | 2245    | 4.04    | 7823.07  | 0.98     |
| 3         | 83344   | 2285    | 4.57    | 7619.01  | 0.98     |

Figure 1 Bacterial community sparse curve

3.2. Distribution of dominant bacteria in garlic wastewater activated sludge

3.2.1. Distribution characteristics of dominant flora at phylum level

At the phylum level, the microbial community distribution and composition of the three samples are shown in Figure 2. The bacterial community structure distribution and composition of the three samples are similar at the phylum classification level. It mainly includes 8 bacteria phyla, Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria, Candidatus Saccharibacteria, Chloroflex, Ignavibacteriae and Parcubacteria, with Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria as the main groups. The relative abundance of the overall bacteria of the four accounts for 80.09%-89.3%. This is consistent with the results of McLellan [4] found that these four main groups are the main bacterial groups of activated sludge from sewage treatment. Bacteroidetes, Proteobacteria, Firmicutes and Actinobacteria all have the function of denitrification, phosphorus removal and degradation of organic matter. On the whole, Bacteroidetes accounted for the largest abundance ratio among the three samples, with an average relative abundance of 39.39%, followed by Proteobacteria, Firmicutes, and Actinobacteria, which reached 25.91%, 10.42%, and 7.45%, respectively. However, the bacterial community abundance of the three samples at the phylum classification level also has some differences. The relative abundance of Bacteroidetes is sample 2>sample 1>sample 3, and the relative abundance of Proteobacteria is sample 1>sample 3>sample 2. The relative abundance of Firmicutes is sample 3>sample 2>sample 1 and the relative abundance of Actinobacteria is sample 2>sample 3>sample 1.
3.2.2. Distribution characteristics of dominant flora at the class level
From the distribution of dominant flora at the class level in Figure 3, there are 7 common dominant classes of the three samples, namely Bacteroidia, Flavobacteriia, Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Clostridia, and Candidatus Saccharibacteria_unclassified. Since garlic wastewater is a high-concentration organic wastewater, the mass concentration of COD is between 5183-5848 mg/L, while Bacteroidia has the function of degrading organic matter and protein [16], and Actinobacteria and Betaproteobacteria have the effect of degrading cellulose. Thus, the three bacteria became the dominant class and dominated by Bacteroidia. The SBR reactor is mainly operated alternately between aerobic and hypoxia during operation, and Clostridia, which has the function of decomposing carbohydrates such as fiber and starch, belongs to the anaerobic flora, which leads to Clostridia becoming the dominant bacteria group. The good NH3-N removal effect of the reactor is due to the combined effect of Candidatus Saccharibacteria_unclassified, Alphaproteobacteria, Betaproteobacteria which have good denitrification functions. Flavobacteriia has a good phosphorus removal function, which is the main reason why the reactor has a better TP removal rate.

Although the three samples shared most of the class layers, the abundance of these common dominant classes had some differences in each community structure. Different samples contain their unique dominant bacteria. For example, the abundance of dominant Anaerolineae in sample 3 is 3.6%, while the abundances in sample 1 and sample 2 are only 0.43% and 0.34%. The main reason for these differences may be the different DO, pH, and sludge concentration in the reactor environment. These factors affect the microbial community structure in the treatment system. [5]

Figure 3 Bacterial community composition at class level
3.2.3. Distribution characteristics of dominant flora at the genus level

The distribution and composition of the microbial communities of the three samples at the genus level are shown in Figure 4. The dominant bacteria genera shared by sample 1 and sample 2 are Macellibacteroides, Fluviicola, Saccharibacteria_genera_incertae_sedis, Petrimonas and Rhodobacter. Because the garlic wastewater contains a lot of protein, polysaccharides and high organic content, and Macellibacteroides and Fluviicola have the function of degrading high concentration organic matter. Petrimonas and Rhodobacter have the ability to produce hydrogen by anaerobic fermentation, and are functional bacteria for anaerobic treatment of high-concentration organic wastewater. The alternate operation of the reactor in anoxic and aerobic environment makes Petrimonas and Rhodobacter the dominant bacteria in the reactor. Due to the high content of NH3-N in garlic wastewater, Saccharibacteria_genera_incertae_sedis, which has a good denitrification function, became the main reason for the dominant bacteria. It is precisely because of the combined action of the above-mentioned dominant bacteria that the COD and NH3-N removal effect of the reactor is above 90%.

However, there are some differences in the community structure of the two samples. The abundances of Macellibacteroides in samples 1 and 2 were 21.48% and 19.23%, respectively; the abundances of Saccharibacteria_genera_incertae_sedis were 1.81% and 11.95%, respectively; the abundances of Fluviicola were 2.44% and 8.82%, respectively; the abundances of Petrimonas in the samples were 2.82% and 4.89%; Rhodobacter abundance is 15.84% and 4.52%, respectively.

It can be seen from Figure 4 that the three samples have similar dominant bacterial genera, but the community structure has certain differences. The abundances of Petrimonas, Fluviicola, Saccharibacteria_genera_incertae_sedis, and Macellibacteroides in sample 3 were 14.17%, 7.15%, 5.85% and 5.6%, respectively. Rhodopseudomonas became the unique dominant genus of sample 3, with an abundance of 5.03%. Rhodopseudomonas is a photosynthetic bacteria that can use various organic compounds as carbon sources for light anaerobic growth. Furthermore, the biofilm where sample 3 is located grows on the upper part of the inner wall of the device, the dissolved oxygen content in the inner wall of the device is less, and the biofilm has been in a micro-aerobic or hypoxic state for a long time. This is the main reason why Rhodopseudomonas became a uniquely dominant genus in sample 3.

4. Conclusion

The activated sludge used to treat garlic wastewater has rich biodiversity, but the bacterial flora structure of different activated sludge samples has certain differences. At the phylum level, the common dominant bacteria phyla of the three groups of samples are Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria, all of which have the function of denitrification, phosphorus removal and degradation.
of organic matter. At the genus level, the dominant genus Macel libacteroides, Fluviicola, Petrimonas, and Rhodobacter have the function of degrading organic matter, and Saccharibacteria_genera_incertae_sedis has the function of removing nitrogen. The removal rate of garlic wastewater pollutants in the reactor is more than 90%, which is the result of the joint action of multiple flora.

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**References**
[1] Jing, C.Y., Ji, X.B., Ling, S.H..(2016) Practice of Garlic Slice Wastewater Treatment Project.China Water & Wastewater, 32(22): 124-127.
[2] Sheng, D.H., Cui F.F..(2017) Isolation and Characterization of an Allicin-degrading Strain. Microbiology China,40(06): 1280 – 1285.
[3] Meng, X.J., Li, H.B., Cao, H.B..(2016) Bacterial Community Composition of Activated Sludge from Coking Wastewater. Environmental science, 37(10): 3923-3930.
[4] McLellan, S.L., Huse, S.M, et al.(2010) Diversity and population structure of sewage derived microorganisms in wastewater treatment plant influent. Environmental Microbiology,12(2):378-392.
[5] Ma, Q., Qu, Y.Y., Shen, W.L., et al. (2015) Bacterial community compositions of coking wastewater treatment plants in steel industry revealed by Illumina high-throughput sequencing. Bioresource Technology, 179: 436-443.