

# AAO-MBR Process for Treatment of Chemical Wastewater and Its Microbial Community

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## Abstract

As a new type of water treatment technology, membrane bioreactor (MBR) has many irreplaceable advantages. Since the separation of SRT (sludge retention time) and HRT (hydraulic retention time), which cannot be achieved by the traditional activated sludge method, can be achieved, the sludge concentration will be greatly improved. In addition, MBR technology also has a small footprint, High denitrification efficiency and other advantages, so it is widely used in water treatment. This paper conducts comparative experiments on AAO-MBR process and AAO process, analyzes the pollutant removal situation and microbial community distribution of the two processes, in order to provide a certain theoretical basis for the promotion and application of AAO-MBR process.

## Keywords

Sewage treatment process, AAO, MBR, microorganism.

## 1. Introduction

In recent years, the country's requirements for sewage treatment plants have become more stringent. GB 18918-2002 "Pollutant Discharge Standards for Urban Sewage Treatment Plants" requires the effluent of sewage treatment plants to meet the first-level A standard: COD 50 mg/L; BOD 10 mg/L; SS10 mg/L; total phosphorus (TP) 0.5 mg/L; total nitrogen (TN) 15 mg/L. If the sewage does not meet the discharge standards, direct discharge can cause eutrophication of lakes and river water bodies. Nitrogen and phosphorus removal in newly-built and existing urban sewage treatment plants is a key concern. An in-depth understanding of the microbial genus diversity and community structure characteristics in sewage treatment plants will help clarify the mechanism of organic matter decomposition and the removal of nitrogen and phosphorus, and provide a microbiological theoretical basis for improving the effectiveness of sewage biological treatment. [1]

At present, there are many sewage treatment plants with AAO process as the main body, and with the gradual maturity of MBR technology, the advanced treatment process of AAO-MBR has gradually been promoted and used, but the structure of the biological community is analyzed at all levels of classification from phylum to genus. There are still few reports. This article takes the activated sludge in the AAO-MBR process and AAO process of a sewage treatment plant in a southern city as the research object. Since the treated sewage contains a small amount of nitrobenzene and aniline pollutants, the effect of nitrobenzene and aniline on the microbial community is explored. influences. Analyze the diversity and community structure characteristics of microorganisms at all levels of classification in sewage treatment plants through high-throughput sequencing technology, observe the community changes of microorganisms in the two sets of processes, with a view to the operation and promotion of the

AAO-MBR process and a small amount of nitrate. The treatment of benzene wastewater provides a microbial basis.

## 2. Materials and methods

### 2.1. Overview of sewage treatment process

The sewage treatment plant is located along the Yangtze River in a town in a city in central Jiangsu. The first phase has a daily processing capacity of 30,000 t/d, including 20,000 t of industrial wastewater and 10,000 t of urban domestic sewage. It was put into operation in 2017 and completed in 2019. See Table 1 for the water quality requirements of the sewage treatment plant. The effluent water quality meets the first level A standard of GB 18918-2002 "Pollutant Discharge Standard for Urban Sewage Treatment Plants".

Table 1 Influent water quality requirements of sewage treatment plants

Pollutants	COD	BOD <sub>5</sub>	SS	Ammonia	Total phosphorus
Industrial water intake limit	≤500mg/L	≤500mg/L	≤500mg/L	≤60mg/L	≤3.0mg/L
Living water limit	≤300mg/L	≤160mg/L	≤180mg/L	≤30mg/L	≤5.0mg/L

### 2.2. High-throughput sequencing

From the anoxic tanks in the AAO-MBR process and the AAO process, 3 points were randomly selected in the aerobic tank for sampling and mixing, each taking 150g. After mixing, a total of 450g of activated sludge was taken out of the two tanks, denoted as A1, A2, B1, B2. Then select 3 points from the MBR tank and the second settling tank for mixed sampling, and mark them as A3 and B3. After sampling, immediately put it in the ice box and bring it back to the laboratory. Use the MIO-BIO PowerSoil DNA Isolation Kit to extract the microbial genomic DNA, and test the integrity of the extracted genome by 1.2% agarose gel electrophoresis. Then sent to the company for Illumina Miseq sequencing.

### 2.3. Analysis of microbial community structure

The PEreads obtained by sequencing first distinguish each sample according to the barcode, then quality control and filter the sequence quality, and then splice according to the overlap relationship. The spliced sequence is again quality controlled and filtered, and finally the optimized sequence is obtained.

Optimize the sequence for OTU cluster analysis and species taxonomy analysis. Based on the results of OTU cluster analysis, multiple diversity index analysis (analysis of alpha diversity within the sample) can be performed on OTU. Calculate the microbial Alpha diversity index, including coverage, richness index, Shannon index, ACE index and Chao1 index. The calculation formula for sample library coverage is  $C=1-N1/N$ , where N1 is the number of OTUs containing only one sequence; N is the total number of sequences. The richness index is calculated by operating the number of taxa to measure the number of species in a single sample. The Shannon index calculation formula is  $H=-\sum P_i \ln P_i$ , where  $P_i$  is the ratio of the number of species in various groups to the total number of species in the sample, and the result can be a measure of community heterogeneity. Both ACE index and Chao1 index are used to estimate the index of

the number of OTUs in the community, and their algorithms are different. The software used for Alpha diversity analysis is Mothur (<http://www.mothur.org/>).

Use RDPclassifier software to classify the sequence of species, select phylum, class, order, family, and genus as taxonomic units, count the number of sequences corresponding to each taxonomy unit, and draw a histogram of species .

### 3. Results and discussion

#### 3.1. Sewage treatment effect

In summer, the sewage temperature is 25~35 °C. According to the operation data of the sewage treatment plant in September 2020, the average value is selected for analysis. The water quality of each section is as shown in Table 2.

Table 2 Water quality of incoming and outgoing water in each section

project		COD /(mg/l)	Total nitrogen /(mg/l)	Ammonia /(mg/l)	Total phosphorus /(mg/l)	Nitrobenzene /(mg/l)
Regulation pool		268	21.9	19.2	0.824	1.23
AAO- MBR	Water ingress	268	21.9	19.2	0.824	1.23
	Effluent	22	7.88	0.0305	0.355	0.211
AAO- Second sink	Water ingress	268	21.9	19.2	0.824	1.23
	Effluent	38	6.53	0.203	0.327	0.269

#### 3.2. Analysis of microbial diversity in sludge

OTU (OperationalTaxonomicUnits) is the same mark set artificially for a certain taxonomic unit (strain, genus, species, grouping, etc.) in order to facilitate analysis in phylogeny or population genetics research. To understand the number of bacteria, genera, etc. in the sequencing results of a sample, it is necessary to sort the sequence (cluster). Through the classification operation, the bioinformatics statistical analysis of the OTUs at 97% similar level can be divided into 1523 classification operation units. Among them, the OTUs of the AAO-MBR process are 1183, 1172 and 1098, and the OTUs of the AAO process are respectively. These are 853, 871 and 826. It can be clearly found that the three stages of the AAO-MBR process are higher than the OTUs in the AAO process, indicating that the number of bacteria in the AAO-MBR process in this experiment is greater than that in the AAO process. The dilution curve is shown in Figure 1.

In order to reflect the abundance and diversity of the microbial flora in the sample, the Alpha index diversity analysis was performed on the obtained sequence, including ACE index, Chao1 index, Shannon index and Simpson index. The results are shown in Table 3, and the coverage rate is above 99%. Which proves that the reliability of this sequencing data is extremely high. The number of bacteria in the AAO-MBR process is greater than that in the AAO process. From

the Chao index and Ace index, the species richness of each reactor of the two processes is the secondary sedimentation tank of the AAO process <the anoxic tank of the AAO process <the aerobic tank of the AAO process <the membrane tank of the AAO-MBR process <AAO -The anoxic tank of the MBR process <the aerobic tank of the AAO-MBR process. The AAO-MBR process has a higher richness. The possible reason is that the membrane treatment reactor uses ultrafiltration technology to remove the macromolecular substances in the sewage. Particles, bacteria, etc. are retained, and then returned to the process tank through sludge return. The sludge return effect is more significant than that of the secondary settling tank in the ordinary AAO process, and it can also significantly reduce the SS in the drainage. From the Shannon and Simpson index, the microbial community diversity in the AAO-MBR process is significantly higher than that in the AAO process. The species diversity of the two aerobic tanks is greater than that of anoxic tanks and membrane tanks. The reason may be that the microbial ecosystems are different under two different operating conditions: anoxic and aerobic. In general, the microbial species abundance and diversity of the AAO-MBR process are better than those of the AAO process.

Table 3 Alpha diversity index of microorganisms in each reaction stage of the two processes

Sample name	coverage	Simpson	chao	ace	shannon
A1	0.997	0.0176	1205.462	1225.635	5.224
A2	0.997	0.0198	1227.844	1240.992	5.171
A3	0.996	0.0263	1134.214	1154.771	4.978
B1	0.995	0.0418	967.271	982.852	4.492
B2	0.996	0.0411	994.079	1008.472	4.483
B3	0.996	0.0431	923.500	936.443	4.428

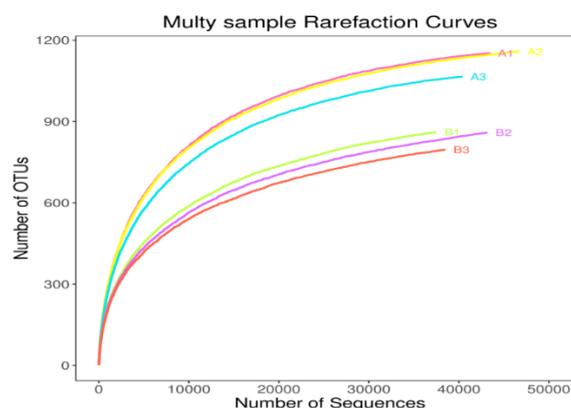


Figure 4 Dilution curve

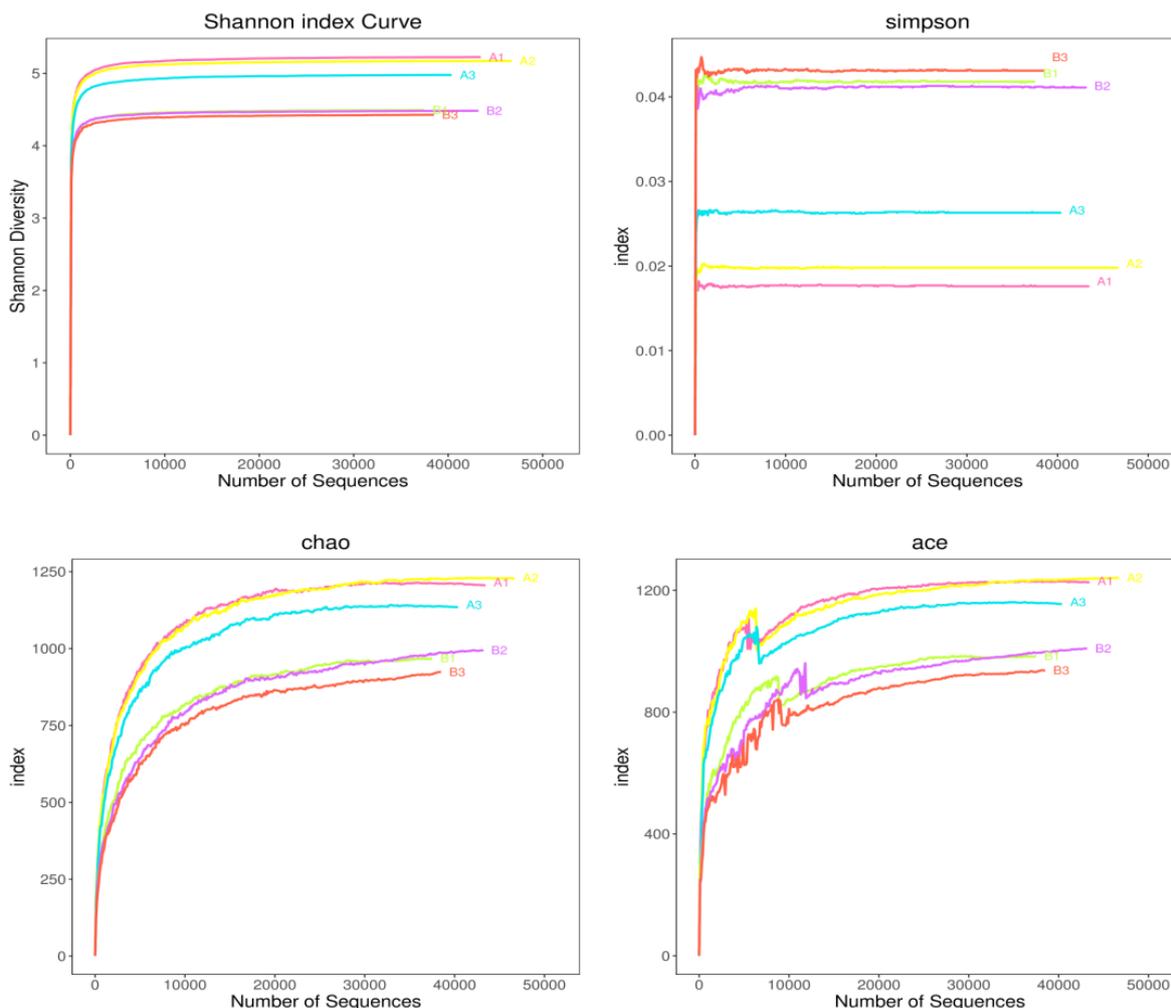


Figure2 Shannon/Simpson/Chao/Ace

### 3.3. Analysis of microbial community structure

The OTU in each sample was classified and statistics by phylum, class, order, family, and genus in order to analyze the population composition in the sample.

#### 3.3.1 Analysis of phylum level community structure

From the community results shown in Figure 3, it can be seen that most of the bacteria are concentrated in the Proteobacteria and Bacteroidetes, which are the first and second dominant bacteria phyla of the two processes, Proteobacteria and Bacteroidetes They are the two larger categories of bacteria in nature. Some denitrifying bacteria and most nitrogen-fixing bacteria also belong to these two categories. Kang Xiaorong's research found that the removal rate of total nitrogen and total phosphorus is positively correlated with the abundance of Proteobacteria and Bacteroidetes. In this experiment, the abundance of Proteobacteria in the AAO process is greater than that in the AAO-MBR process, but the abundance of Bacteroidetes, Planctomycetes and Acidobacteria in the AAO-MBR process is greater than that in the AAO process. It can be clearly seen from the above figure that the microbial community structure in the AAO-MBR process is more diverse.

Acidobacteria are obligate acidophilic bacteria. Studies have shown that some members of the Acidobacteria are involved in the metabolism of single-carbon compounds, degrading cellulose under micro-aerobic and hypoxic conditions to produce acetic acid and hydrogen. Firmicutes are gram-positive bacteria that account for 2.73% of the wastewater treatment plant. They can be metabolized in an aerobic or anoxic environment, and are mainly involved in the process of

nitrification and denitrification. Bacteroidetes are obligate anaerobic microorganisms, accounting for 2.24%. Research data shows that the Bacteroides phylum is beneficial to promote the utilization of nitrogenous substances, biotransformation of steroids and hydrolysis of macromolecular substances. Therefore, it is speculated that the Bacteroides phylum plays an important role in the process of biological denitrification.

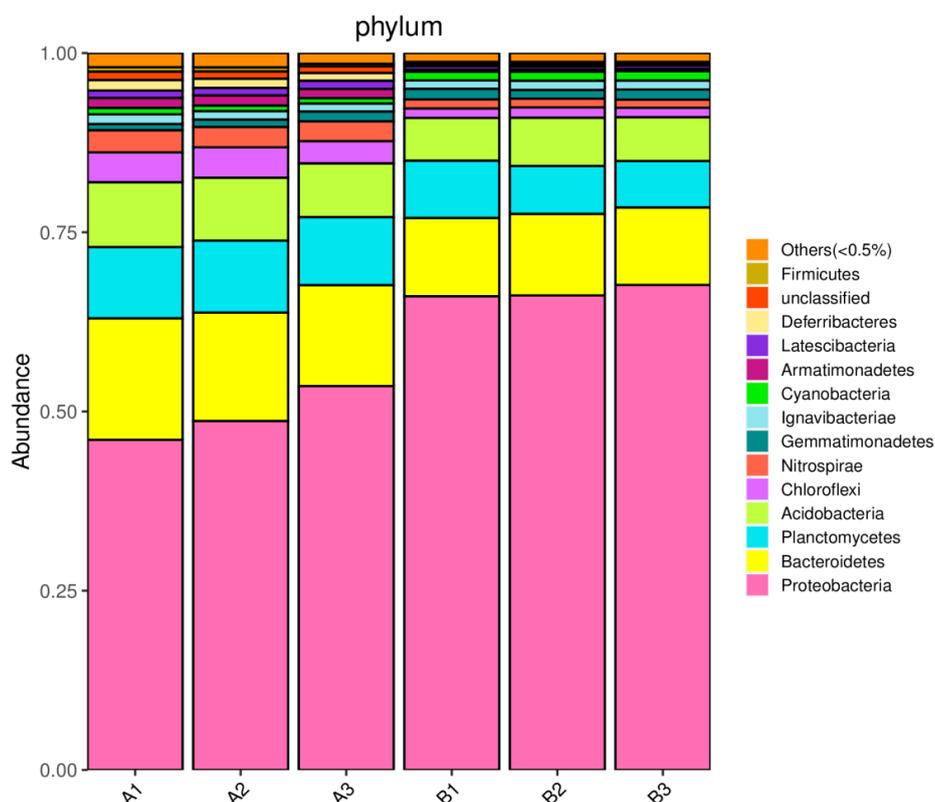


Figure 3 Gate level community structure

### 3.3.2 Analysis of class level community structure

The analysis results of activated sludge samples at the "class" level are shown in Figure 7. In the two processes, Betaproteobacteria is the most abundant class system, accounting for 23.6% and 37.1 respectively. %. Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria belong to the phylum Proteobacteria, so it can be seen that at the class level, Proteobacteria bacteria are dominant in both processes.  $\alpha$ -Proteobacteria are autotrophic microorganisms, some of which play an important role in the nitrification process.  $\beta$ -Proteobacteria and  $\gamma$ -Proteobacteria are mostly facultative hetero aerobic bacteria, which use organic matter as a carbon source. They are the main participants in the degradation of COD in the sewage treatment system and are the most active sludge microorganisms in domestic sewage treatment plants. An important outline.  $\delta$ -Proteobacteria include some species that are strictly anaerobic, and they also have the function of degrading COD.

### 3.3.3 Analysis of horizontal community structure

In the 6 samples in this sequence, the relative abundance of the microbial communities in each pond is shown in Figure 4. The proportion of Unclassified in each pool is 38.76%~59.89%, and the relative abundance in the AAO-MBR process is much greater than that in the AAO process. Nitrospira, an important genus of nitrifying bacteria, was detected in the two processes, and the average content in the two processes was 2.9% and 1.6%, respectively. Dechloromonas, Nitrospira, and Stenotrophobacter are the dominant bacteria in the AAO-MBR process anoxic

tank; Dechloromonas, Denitromonas, and Nitrospira are the dominant bacteria in the AAO-MBR process and aerobic tank and membrane tank; Dechloromonas, Denitromonas, and Thiogranum are the lack of AAO process The dominant bacterial genera in the oxygen tank; Dechloromonas, Denitromonas, Thiogranum, and Defluviococcus are the dominant bacterial genera in the AAO process aerobic tank; Dechloromonas, Denitromonas, Thiogranum, and Thauera are the dominant bacterial genera in the AAO process secondary sedimentation tank.

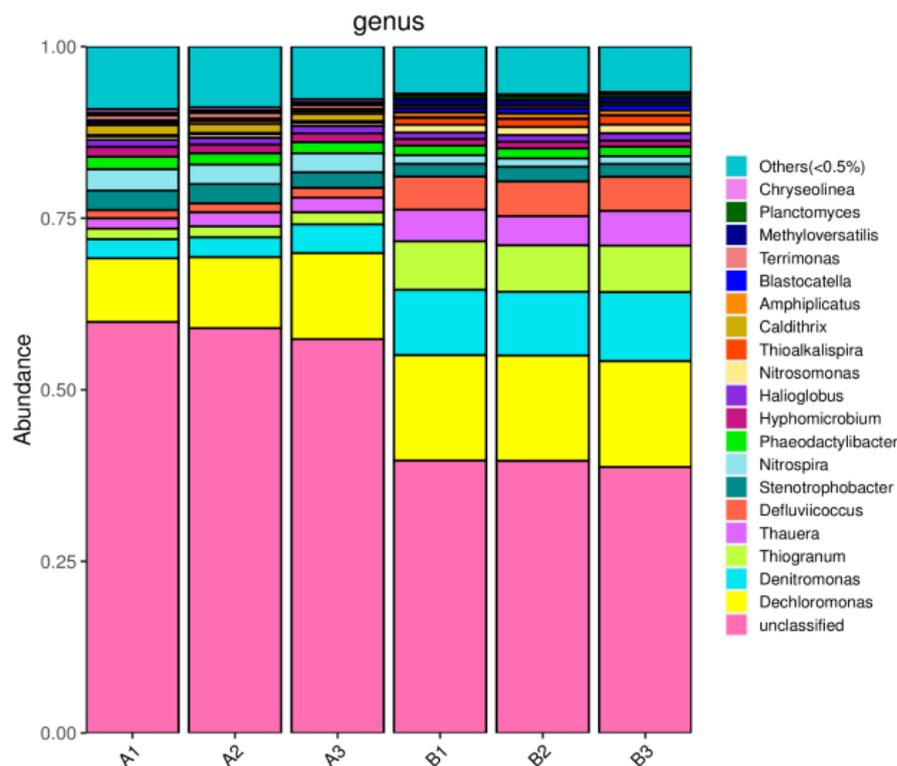


Figure 4 is a horizontal community structure

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