






Comparative Analysis on Microbial Characteristics of Aerobic Activated Sludge and Biofilm in Low-temperature A²O Process

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Abstract: In the low-temperature A²O coupling system, activated sludge and biofilm perform corresponding denitrification and phosphorus removal functions through the growth of specific microorganisms. The difference of microbial populations under different growth conditions is of great significance to the wastewater treatment effect. In this study, high-throughput sequencing technology was used to analyze the population differences of suspended activated sludge and attached biofilms in aerobic cells in the same environment, in order to analyze the pollutant removal mechanism. The results showed that the abundance and diversity of aerobic biofilm fillers were higher than that of aerobic activated sludge, the microorganisms in both biofilm and activated sludge mainly degrade organic matter. Both activated sludge and biofilm accumulated and domesticated a variety of high-abundance denitrification and phosphorus removal bacteria, but the distribution abundance of different bacteria in the two regions were different. The biofilm was rich in many denitrifying bacteria *Dokdonella*, *Denitratisoma*, and phosphorus-accumulating bacteria *Candidatus_Accumulibacter*, and also enriched in *Bacteroidetes_vadinHA17* with anaerobic phosphorus removal function; While, the activated sludge contained more *Cloacibacterium*, *Lactococcus* related to sugar metabolism, and enriched phosphorus accumulating bacteria *Tetrasphaera*, *Candidatus_Accumulibacter*, and aerobic denitrifying bacteria *Phreatobacter*, *Aeromonas*.


1 INTRODUCTION


Biological treatment technology is widely used in urban sewage treatment plants as a sewage purification process with relatively low cost and good treatment effect. The microorganisms in the treatment system mainly achieve sewage purification by metabolizing and decomposing organic pollutants in the sewage (Zhang 2021). Therefore, the level of microbial metabolic activity in the system directly affects the sewage treatment effect. Among the environmental impact factors, temperature is an important factor (Dai 2020). In northern China, the low temperature period in winter is long, which


seriously affects the degradation efficiency of pollutants in the sewage treatment system (Shang 2020, Duan 2016). However, in practical engineering and research, it has been found that the biological treatment system still has a certain removal effect on pollutants at low temperatures, mainly because the cold-adapted microorganisms in the system play a role (Shang 2020, Luo 2020). Therefore, the microbial ecology analysis of the changes in the microbial community structure and the main functional flora and typical flora in sewage treatment will help explain the pollutant removal mechanism, thereby providing theoretical guidance and technical support for the application of biological improvement measures for low-temperature operation.


In the low temperature environment in winter, the performance of nitrification and phosphorus removal is significantly reduced, and it is difficult for a single treatment technology to make the

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effluent of the sewage treatment plant reach the sewage treatment standard. Microorganisms grow in suspension in activated sludge and adhere to the biofilm. Therefore, there is a big difference in the groups of microorganisms that grow in two different growth environments. The biofilm-activated sludge coupling process adopts the combination of activated sludge method and biomembrane method to make up for the shortcomings of a single process, which can effectively improve the removal effect of pollutants and improve the stability of the system (Ai 2021, Zhang 2021, Wang 2018). Studies have found that the addition of fillers can effectively improve the simultaneous removal of nitrogen and phosphorus by the multi-stage AO process on domestic sewage with a low carbon to nitrogen ratio (Wang 2020).

In this study, high-throughput sequencing was performed on the activated sludge and biofilm samples in the aerobic zone of the biofilm-activated sludge coupling A²O process, which worked well at 15°C. The differences in the community abundance and structure of microorganisms growing in suspension in activated sludge and adhering to biofilms were analyzed to explain the removal mechanism of pollutants in urban low-temperature sewage treatment.

2 MATERIAL AND METHOD

2.1 The Reactor Devices

The experiment used a modified A²O reactor: the aerobic zone was split into two parts of the same volume; each part was filled with drifting balls, the filling rate was 30%. The total reactor volume of was 48 L, and the effective volume was 42 L, divided into four tanks, and along the length of the reactor were A₁, A₂, O₁, and O₂ tanks. The volume ratio of the anoxic tank to the aerobic tank of the system reactor was 3:4; the interior of each tank was filled with dispersing balls, and the inside of the balls were equipped with polyurethane sponge and Pall ring, the filling rate was 30%, and each anaerobic tank was equipped with a stirrer Device.

2.2 Reactor Operating Conditions

The operating temperature was 15°C, simulating low-temperature urban sewage. Adopting the segmented water inlet method, the ratio of inlet water flow rate was A₁:O₁=2:1, water inflow Q=84 L/d,

HRT=12 h, SRT=15 d, sludge reflux ratio 100%, and the nitrification liquid reflux ratio was 200%.

2.3 Pollutant Removal Condition

The synthetic wastewater used in this experiment simulated domestic sewage, including peptone, beef extract, soluble starch, NH₄Cl, KH₂PO₄, CH₃COONa, NaHCO₃, etc. The experiment water quality and effluent water quality when the reactor was operated for 15 days were shown in table 1.

Table 1: Pollutant removal

water quality index	Concentration range of influent (mg/L)	Mean concentration of effluent (mg/L)	Average removal rate
COD	246.80~328.70	33.36	90.9%
NH ₄ ⁺ -N	30.43~32.48	2.87	89.1%
TN	38.43~42.12	9.16	77.2%
TP	3.48~3.52	0.07	98.0%

2.4 High-Throughput Sequencing

Total genomic DNA of the biofilm and activated sludge samples were extracted using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions.

The two-step PCR method was used to amplified the extracted DNA, and the sample-specific 16-bp barcode was incorporated into the forward and reverse primers for multiple sequencing in the second step of PCR. The total PCR amplicon was purified by Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using PicoGreen dsDNA detection kit (Invitrogen, Carlsbad, CA, USA). Finally, pair-end sequencing was performed using the NovaSeq sequencer platform from Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The analysis was conducted on their online platform.

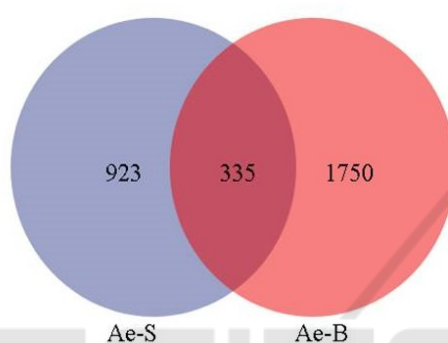
3 RESULTS AND DISCUSSIONS

3.1 Analysis of Microbial Abundance and Diversity

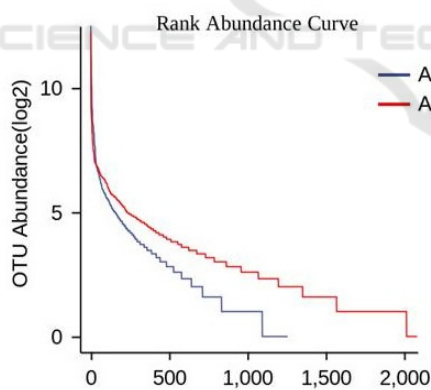
The high-throughput sequencing results of activated sludge and biofilm samples in aerobic cells showed

that the effective sequencing amounts of aerobic sludge (Ae-S) and aerobic biofilm filler (Ae-B) were 75235 and 63873, respectively. The number of OTU clusters of aerobic sludge and biofilm filler were 1258, 2085; 335 OTUs were owned by both, unique OTUs for aerobic activated sludge were 465, and unique OTUs for biofilm were 697, represented by Venn diagram as shown in Figure 1a. There were much more microorganisms enriched in biofilm.

The OTU Rank diagram showed that the curve of biofilm was wider and flatter, which indicating there were more abundant microbial species and higher uniformity in the biofilm (Fig. 1b). The results above indicated that biofilm filler can enrich more kinds of microorganisms and increase the biomass of the system.



(a) Venn diagram



(b) OTU rank diagram

Figure 1: Diagram of OTU distribution.

3.2 Analysis of Taxonomic Composition

The microorganisms distribution in the aerobic sludge and biofilm at different taxonomic level was shown in Table 2. There were 22 phyla, 49 classes, 114 orders, 177 families and 263 genus microorganisms detected in the aerobic sludge

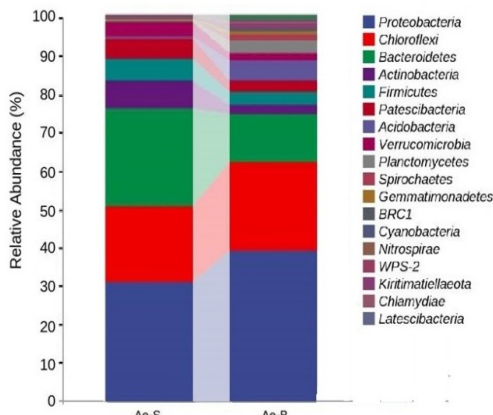
sample; and 40 phyla, 85 classes, 177 orders, 277 families and 372 genus were detected in the biofilm sample.

Table 2: Different levels of microbial taxa statistics

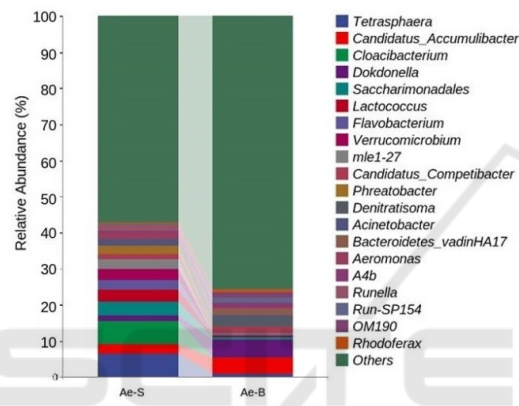
ID	phylum	class	order	family	genus
Activated Sludge	22	49	114	177	263
Biofilm	40	85	177	277	372

The function of microorganisms largely depends on the community structure of microorganisms. The distribution the top 20 phyla with relative abundance of samples in the sludge and biofilm of aerobic tank was shown in Figure 2a. The results showed that Proteobacteria had the highest abundance both in the sludge and biofilm, followed by Bacteroidetes and Chloroflexi in sludge, but there was more Chloroflexi than Bacteroidetes in biofilm. The trend of dominant bacteria at the phylum level for the two samples was different.

The Figure 2b showed the top 20 bacterial genus with relative abundance in the aerobic system. The distribution of dominant bacteria at the genus level in the sludge and biofilm of aerobic tank were also very different as shown in Figure 2b. In activated sludge, the bacteria that mainly related to sugar metabolism (Han 2020) were *Cloacibacterium* (6.38% of total sludge bacteria), *Lactococcus* (3.18%); the bacteria that had phosphorus removal function (Marques 2017) were *Tetrasphaera* (6.33%), *Candidatus_Accumulibacter* (2.69%), *Acinetobacter* (1.78%); the genus of bacteria with denitrification function were *Flavobacterium* (2.72%), *Phreatobacter* (2.53%), aerobic denitrifying bacteria *Aeromonas* (1.78%), *Dokdonella* (1.65%). The genus that playing roles in aerobic biofilm were quite different from activated sludge. The genus with sugar metabolism was *Candidatus_Cometibacter* (1.67% of total biofilm bacteria); the genus that playing roles in phosphorus removal were *Candidatus_Accumulibacter* (4.42%), *Bacteroidetes_vadinHA17* (2.02%) with the function of anaerobic dephosphorization (Hao 2020); The genus responsible for denitrification were *Dokdonella* (4.80%) and *Denitratisoma* (2.55%). The results showed that there were more long-generation denitrification functional flora and some other functional flora enriched in biofilm.



(a) phylum



(b) genus

Figure 2: Diagram of taxonomic composition.

3.3 Genus Composition Heat Map

In order to further compare the differences in species composition between activated sludge and biofilm samples, the data of the top 20 species with average abundance was used to draw a genus composition analysis heat map (Fig. 3). In the activated sludge had gathered some bacteria related to flocculation, such as *Saccharimonadales*, some aerobic denitrifying bacteria, such as *Aeromonas*. And the biofilm enriched with some anaerobic functional microorganisms, such as *Bacteroidetes_vadinHA17* had been reported to have the function of anaerobic dephosphorization (Cho 2018), *RBG-13-54-9* was low-temperature dominant bacteria for phosphorus removal (Mei 2020). Under different carrier environments, the distribution of genus-level dominant bacteria in aerobic activated sludge and biofilm was very different. This may be related to the fact that the biofilm carrier could construct a more complex environment.

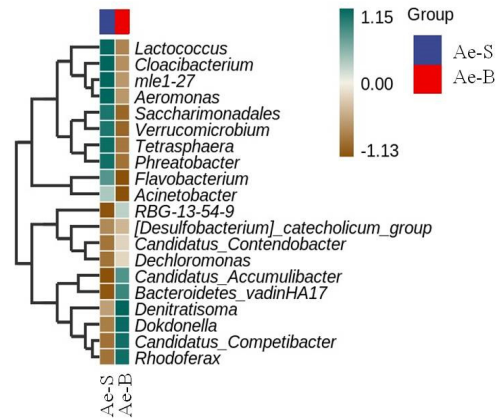


Figure 3: Diagram of genus composition heat map.

4 CONCLUSIONS

Although other conditions were the same, the results showed that the abundance and diversity of microorganisms on the biofilm were more than that of activated sludge. The biofilm-activated sludge coupling system increases the biomass in the environment by creating more complex organisms due to the addition of carriers. The biofilm was enriched with more long-generation denitrification functional flora, such as *Dokdonella* and *Denitratisona*, so adding biofilm to the activated sludge system is more conducive to enhancing the denitrification efficiency.

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