

Research on the Effects of Sewage Discharge from Chemical Industry Park on Microbial Community

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To analyze structure change of microbial communities in a sewage system of a typical coastal industrial park and a drainage area, by comparing structures of microbial communities in the sewage treatment system in the park and the pollutant-holding area by means of molecular biology, higher richness and diversity of bacteria and archaea in sediments in the pollutant-holding area than that of the sewage treatment system in the park are found in this research; according to analysis and findings on different points and different seasonal samples in the pollutant-holding area, microbial community structures at each point in the pollutant-holding area share relatively high similarity. Sediments have higher bacterial community structure diversity than water body, but their diversity of archaeal community structure is similar. Conclusion are drawn through experiments as follows: AOA and AOB sediments have higher activity under relatively lower (<0.1mg/L) and higher (>2mg/L) concentration of ammonia nitrogen; ammonia oxidation capacity in the sediments is increased during the process where the temperature rises from 5°C to 35°C; increase of salinity can make the activity of AOA improve and make it become the main ammonia oxidization bacteria flora.

1. Introduction

1.1 Research significance

With a typical coastal industrial park chosen as the research object, intermittent sampling and environmental monitoring are carried out on the sewage treatment system in the park and the sewage discharge area where it is located, meanwhile (Harris et al, 2017), quantitative PCR, clone library, 454 high throughput sequencing and molecular biology methods are adopted for analysis of changes of microbial community structure of the sewage treatment system in the industrial park and of the pollutant-holding area to study response relationship between microbial community structure in the bay area and environmental factors, and to explore the influence which may be caused by terrigenous industrial pollution on the microbial community structure in the pollutant-holding bay area (Henson, 2014). In view of serious pollution of inorganic nitrogen, special attention is paid to the change of ammonia oxidization bacteria flora in microbial community and to investigation on the influence of industrial sewage discharge on ammonia oxidization bacteria flora in sewage receiving area. Implementation of the research can enrich and deepen the understanding on the microbial community structure in the industrial sewage treatment system and in the industrial pollution-holding bay, providing practical accumulation for mastering the impact of industrial pollution input on the marine ecosystem polluted by pollutants (Jiang et al., 2013).

1.2 Research contents

Real-time PCR, clone library (Li et al., 2000), 454 high-throughput sequencing and other molecular biological methods are mainly adopted in the research to study the microbial community structure in the sewage treatment system of the typical coastal industrial park and in the pollutant-holding area with focuses on analysis of the ammonia oxidation microbial community and exploring impacts of the industrial pollution input on microbial community structure in pollutant-holding sea area (Li et al., 2017).

At the same time, through construction and culture of a laboratory micro-environment simulation system (Liao et al., 2014), the influences of ammonia, temperature, salinity and other environmental factors on the

ammonia oxidization microbial community in the pollutant-holding sea area are investigated (Manukyan et al., 2012). The main research contents are as follows:

(1) Take a typical coastal industrial park and its pollutant-holding area as research objects, seasonally collect samples from the typical sewage treatment system and its emission point, analyze their microbial community structure, and compare the similarities and differences between those two (Moctezuma-Cantorán I., et al., 2012).

(2) Set up sampling sites at different locations of the sewage discharge sites in the park, seasonally collect water at each sampling point and samples from sediments, analyze the microbial community structure (Nguyen et al., 2015), and investigate the response relationship between environmental factors and the microbial community structure.

(3) The simulation system of seawater-sediment micro environment is constructed in the laboratory (Pitt, 2000). The effects of environmental factors such as ammonia nitrogen, temperature and salinity on the structure and activity of ammonia oxidization bacteria in pollutant-holding area are studied by the laboratory simulation and culture (Qiu et al., 2015).

2. Experiment contents

2.1 Comparison of microbial community structure between the sewage treatment system and the sewage discharge point

Shangyu Sewage Treatment Plant (SY), the State Pharmaceutical (GB) and Yuntao Biotechnology (YT) were chosen, and activated sludge or sediment samples on the discharge outlet were used for researched with the samples marked as SY-Feb, SY-Aug, GB-Feb, GB-Aug, YT-Feb, YT-Aug, HZW-Feb and HZW-Aug.

Method:

(1) Total DNA was extracted from the selected activated sludge and sediment samples;

(2) The bacterial 16SrRNA gene, archaeal 16SrRNA gene, bacterial amoA and archaeal amoA in the total DNA of the samples were quantitatively determined by using selected primers;

(3) For bacterial 16SrRNA gene and archaeal 16SrRNA gene, high throughput sequencing was used to analyze diversity of bacteria and archaea cultivated for two months;

2.2 Temporal and spatial variation of microbial community structure in pollutant-holding area

The samples in the pollutant-holding area were selected and analyzed with the sediment samples numbered as follows: S2-May, S2-Aug, S2-Nov and S4-Feb.

Method:

(1) DNA was extracted from the selected sediment samples and water samples W4Feb, W5Feb;

(2) Quantitative determination on bacterial 16SrRNA gene, archaeal 16SrRNA gene, bacterial amoA and archaeal amoA was carried out with DNA samples in sediments;

(3) Diversity analysis on 16SrRNA gene and archaeal 16SrRNA gene in sediments and DNA samples in two water bodies was carried out with 454 high throughput sequencing;

(4) Diversity of AOBamoA and AOAamoA of DNA samples in sediments was analyzed with 454 high flux sequencing.

2.3 Effect of environmental factors on structure and activity of ammonia oxidization bacteria in pollutant-holding area

Experimental design:

There were six ammonia nitrogen concentration levels in the simulated ammonia culture experiment. The culture system was constructed in a 120ml cone flask. 10g sediment samples and 50ml water samples were added to each system, sealed with gas permeable membrane, and placed in a 25°C dark environment for stationary culture.

A total of 4 temperature levels which were 5°C, 15°C, 25°C and 35°C separately were set in the simulation culture experiment of temperature factors. The culture system was constructed in a 120ml cone bottle. 10g sediment samples and 50ml water samples were added into each system, sealed with breathable sealing films, and placed under various design temperature conditions for light tight culture.

There are, in the simulation cultivation experiment of salinity factors, 3 salinity levels which were 5psu, 15psu and 120ml respectively. 30psuo culture system was constructed in a 120ml conical flask with 10g sediment samples and 50ml water samples added into each system. The water salinity was adjusted to the design level by adding exogenous NaCl, and it was sealed with breathable sealing membranes and placed in 25°C dark

environment for stationary culture. Oscillation was carried out on the culture system by way of manual operation once a day to exchange oxygen.

3. Result and analysis

3.1 Diversity of bacterial community

It can be seen from the dilution curve (Figure 1) that the bacterial community richness in sediment samples is higher than that in activated sludge samples.

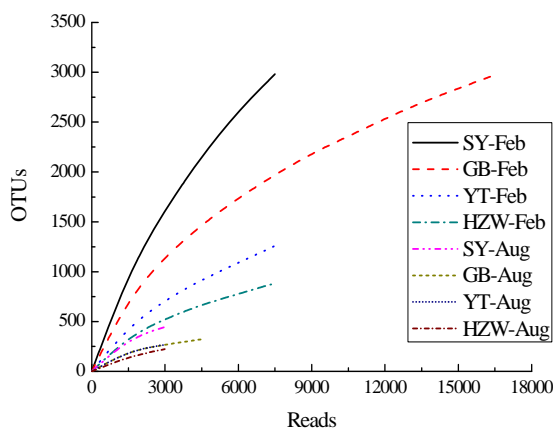


Figure 1: Bacterial 16S rRNA gene library dilution curve

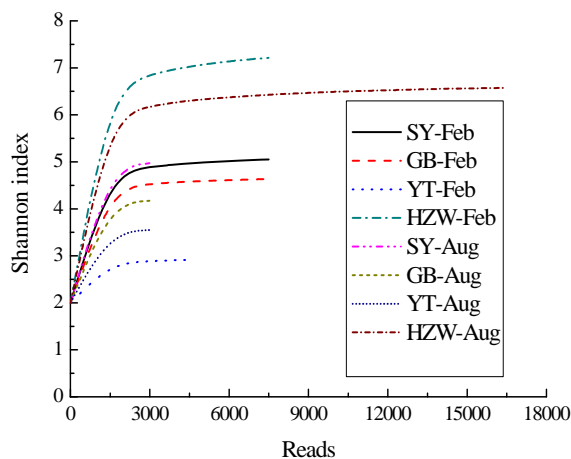


Figure 2: Bacterial 16S, rRNA gene library, Shannon index curve

It can be seen from the Shannon index curve (Figure 2) that the bacterial community of the sediment samples has higher biological diversity than that of the activated sludge samples.

3.2 Spatial and temporal variation of bacterial community structure

The bacterial 16SrRNA gene library is constructed by 454 high-throughput sequencing of all four sediment samples and two water samples—W4-Feb and WS-Feb. A total of 131526 effective sequences are obtained, and the statistics of each library are shown in Figure 3.

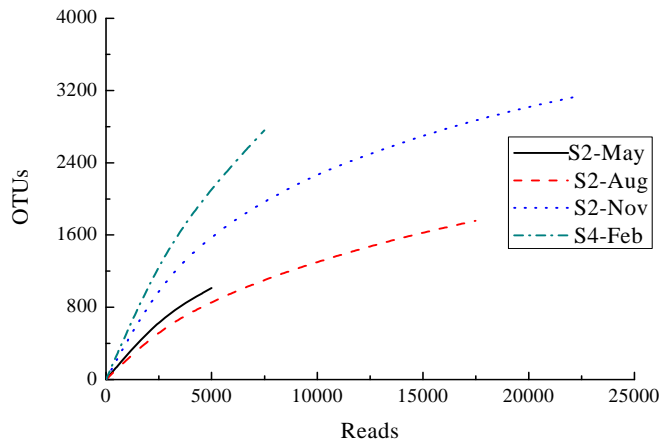


Figure 3: Bacterial 16S rRNA gene library dilution curve

3.3 Effects of ammonia nitrogen concentration, temperature and salinity in water body on the structure and activity of ammonia oxidization bacteria in sediments

During the period of culture, the amount of ammonia nitrogen added to the culture system of each ammonia nitrogen concentration level is shown in Chart 4.4, where the ammonia nitrogen concentration in the AO group after culture is lower than the detection limit (0.01mg/L) of the test method. After 56 days of culture, the water quality of the culture systems with different ammonia nitrogen levels is shown in Chart 4.4.

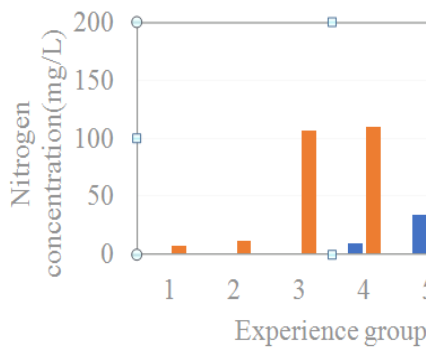


Figure 4: The different concentration of ammonia after water quality training system

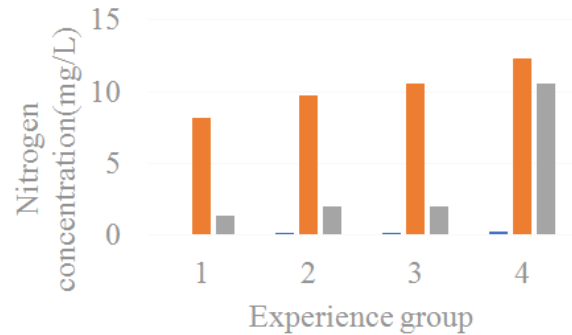


Figure 5: Simulation results of temperature factors

It can be seen that nitrate is produced in the culture systems with each ammonia nitrogen concentration, which indicates that nitrification occurs in all the systems during the culture process.

After 56 days of culture, the water quality in different temperature culture systems and PNR in sediments are shown in Figure 5.

It can be seen that nitrate is produced in each culture system, that nitrate nitrogen concentration is 8.04mg/L-12.11mg/L, that nitrite nitrogen accumulation is relatively little with the concentration as 0.01mg/L-0.09mg/L, which indicate that nitrification occurs with good effects in each culture system. Accumulated amount of nitrite and nitrate increases with the increase of culture temperature, indicating that the nitrification capacity of the culture system increases with the increase of temperature.

After 56 days of culture, the water quality in different salinity culture systems and PNR in sediment are shown in Figure 6.

It can be seen that nitrate is produced in each culture system, that nitrate nitrogen concentration is 9.89mg/L-11.90mg/L, that nitrite nitrogen accumulation is relatively little with concentration as 0.02mg/L-0.03mg/L, which indicate that nitrification occurs with good effects in each culture system.

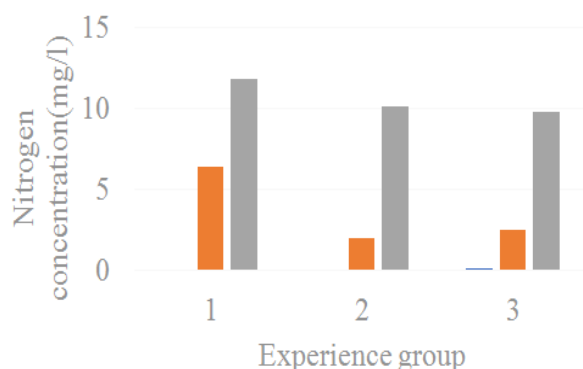


Figure 6: Experimental results of salinity factors

4. Conclusion

Shangyu Industrial Park and its pollutant-holding area are selected in the research as the research objects, while seasonal sampling analysis is used to analyze the microbial community structure of sewage treatment system in the park and in the pollutant-holding area. At the same time, the microbial community structure in different seasons in the pollutant-holding area is analyzed in this research which is the first detailed research on the microbial community structure. In addition, the effects of ammonia nitrogen, temperature, salinity and other environmental factors on ammonia oxidization bacteria structure in the pollutant-holding area are analyzed in the research through the simulation culture experiments in the laboratory, where research on the influence of ammonia nitrogen factors reveals activity change rules of AOA and AOB under different ammonia concentration levels in the water environment. The main conclusions of the research are as follows:

(1) Activated sludge in the sewage treatment system has higher biomass than sediments in the pollutant-holding area, at the same time, the number of bacteria in two kinds of samples are higher than those of archaea; as for richness and diversity of bacteria and archaea, sediments in pollutant-holding area are higher than activated sludge samples in the sewage treatment system. The flow direction of sewage promotes higher similarity regarding bacterial community structure in the two water environments, but its influence on the archaeal community structure is not obvious.

(2) Seasonal variation of microbial community structure in the pollutant-holding area is obvious, while microbial community structure at each point in the pollutant-holding area remains relatively high similarity. The sediments show a higher diversity of bacterial community structure than water, and contain most of the bacterial species in the water samples. However, diversity of the archaeal community structure is consistent with that of the water body.

(3) The amount of AOA in the sewage treatment system is very low, while AOB is the main body of ammonia oxidation. The ratio of AOA and AOB in sediments of the pollutant-holding area is 0.39-5.52, which means they are close with regard to quantity. The influence of terrestrial pollution input on AOB community structure in pollutant-holding area is more obvious than that on AOA community structure.

(4) The proportion of AOA in archaea is higher than that of AOB in bacteria, and the increase of ammonia nitrogen concentration in water can promote the increase in proportion of ammonia oxidization bacteria in microbial community in sediments and increase the proportion of AOA in ammonia oxidization bacteria. Ammonia, pH, nitrate nitrogen and temperature in water show negative correlation with Nitrosomonas-like cluster in AOB in sediments, while COD, DO and salinity in water show a weak positive correlation with Nitrosopumilus cluster in AOA.

(5) Under the poor ammonia nitrogen condition, ammonia oxidation activity of AOA (as the main flora in ammonia oxidation) in sediments is higher than that of AOB; while high ammonia nitrogen concentration can greatly stimulate the ammonia oxidation activity of AOB, making it become the main body of ammonia oxidation in the sediments, the ammonia oxidation activity of AOA is inhibited under the condition of high ammonia concentration.

(6) The ammonia oxidation capacity of sediments in the pollutant-holding area is improved when the culture temperature is increased from 50C to 350C.

(7) The response of AOB in sediments of the pollutant-holding area to salinity of the water body is not obvious, while the higher salinity water environment is more suitable for growth of AOA, which makes AOA show higher ammonia oxidation activity in the high salinity environment, and becomes the main ammonia oxidization bacteria flora.

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