Effect of C/N on removal of chlorpyrifos in constructed wetland system

Zhiyu Fan¹, Xuefei Sun², Yue Quan¹, Weihong Zhu², Mingji Jin*¹

¹ Department of Resource utilization and plant protection, Yanbian University, Yanji, Jilin, 133002, P.R. China
² Department of Physical geography, Yanbian University, Yanji, Jilin, 133002, P.R. China
*Corresponding author’s e-mail: jinmingji@ybu.edu.cn

Abstract. This study used C/N as a variable to investigate the removal effect of chlorpyrifos and the microbial community structure in the constructed system. As a result, with the increase of C/N within the range of 2-20, the removal rate of chlorpyrifos, the system matrix and the concentration of chlorpyrifos in plants all increased. The diversity of microbial community in the system was significantly affected by C/N. When C/N was 5, the microbial community diversity was the most abundant. Proteobacteria and Alphaproteobacteria were the dominant phylum and class.

1. Introduction

Constructed wetland system is an ecological treatment system for purifying sewage through the coordination of physics, chemistry and biology. It is widely used in the field of sewage treatment because of its small investment, easy management and low operating cost. C/N is the main factor affecting the activity of microorganisms[1-2]. In the constructed wetland system, C/N affects the ability of the system to treat pollutants through microbial activity[3]. Therefore, this study uses C/N as the operating condition to explore the effects of different C/N on the removal of chlorpyrifos[4] in constructed wetland systems, in order to provide a basis for the application research of constructed wetland systems.

2. Experimental materials and methods

2.1 Experimental setup

As shown in Figure 1, the experimental device was made of a plastic bucket, and a water collecting pipe was buried at the bottom of the device, which was filled with 2 cm gravel and 15 cm mixed soil. In the experiment, the water surface was maintained 2 cm above the substrate, the calamus was evenly planted in the system, and the planting density was 212 plants per m².
2.2 Experimental design

In the experimental, C/N was a variable to raise from 2 to 5, 10, 15 and 20 successively, in order to investigate the removal effect of chlorpyrifos and the changes of microbial community structure. The experiment used surface flow constructed wetland and intermittent operation mode. Each group of experiments was run for 7 days and repeated 5 times. The experimental water was artificially simulated wastewater, and the chlorpyrifos was dissolved in water. The initial concentration of chlorpyrifos was 0.96 mg/L.

2.3 Method of analysis

At the end of one experiment period, the water and soil samples were taken to measure the concentration of chlorpyrifos, and the experimental results were taken as the average of 5 replicates, the chlorpyrifos test plant samples were sampled after 5 repeated experiments, and the chlorpyrifos was determined by ultraviolet spectrophotometry [5-8]. Soil samples for microbial analysis with C/N values of 5, 10, 15 and 20 were collected at the end of the 5 repeated experiments. The specific numbers were C1, C2, C3 and C4, and the microbial community structure was analyzed using high-throughput sequencing[9-11].

3. Results and discussion

3.1 Chlorpyrifos removal effect

As shown in Figure 2, the removal effect of chlorpyrifos under different C/N conditions in the system. With the increase of C/N, carbon source was sufficient in the system, providing sufficient nutrients and energy basis for microbial growth and metabolism, and improving microbial activity[12]. Moreover, the increase of carbon source can stimulate the microbial to secrete more chlorpyrifos degrading enzyme[13]. Therefore, the removal effect of chlorpyrifos showed an increasing trend, with highest removal rate was 92.0% when C/N was 20.

In the constructed wetland system, the pollutants were also removed by substrates adsorption and plant absorption. Therefore, the concentration of chlorpyrifos in the substrates and in the plant was analyzed in this study. As shown in Figure 3, the concentration of chlorpyrifos in the substrates at different C/N conditions. With the increase of C/N, the microbial activity was enhanced and the chlorpyrifos degrading enzyme was increased in the system, so that chlorpyrifos which can be degraded and absorbed by microorganisms and plants could be better fixed in the substrates surface,
and the concentration of chlorpyrifos in the substrates increases. Therefore, with the increase of C/N, the concentration of chlorpyrifos in plants also increased due to the increase of carbon source, enhanced plant photosynthesis and vigorous growth.

![Figure 2: Chlorpyrifos removal effect](image)

![Figure 3: Substrates and chlorpyrifos concentration in plants](image)

### 3.2 Microbial community structure analysis

As shown in Table 1, high-throughput sequencing results show that the system microbial community richness (Chao 1 and ACE) was C1>C2>C4>C3, and the uniformity (Shannon and Simpson) was C1>C2>C4>C3. When C/N was 5, the microbial community diversity was the most abundant, while the C/N increasing system was mainly dominated by organic degradation microorganisms, and the diversity was also reduced. The difference of microbial community diversity among the samples was significantly (p<0.01), indicating that C/N was one of the factors that change of microbial community diversity.

| Samples | OTUs   | Simpson | Shannon | Chao1    | ACE      |
|---------|--------|---------|---------|----------|----------|
| C1      | 47468  | 0.999   | 11.05   | 4168.1   | 4174.6   |
In order to visualize the abundance of each sample at different levels of microbial classification, the microbial community of each sample were analyzed at phylum and class. The results are shown in Figure 4. The higher relative abundance of the top seven predominant bacterial phyla were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Firmicutes, Bacteroidetes and Gemmatimonadetes, and the relative abundance was greater than 90%. Among them, the relative abundance of Proteobacteria was the highest in each sample. In the range of C/N from 5 to 15, Firmicutes and Bacteroidetes increase with the increase of C/N, indicating the dominant bacteria in the system under high C/N conditions, while the abundance of Actinobacteria and Chloroflexi decreases, which was dominant bacteria under low C/N conditions in the system.

As shown in Figure 5, The higher relative abundance of the top six predominant bacterial classes were Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Subgroup_6, Deltaproteobacteria and Clostridia. and the relative abundance was more than 50%. Among them, the relative abundance of Alphaproteobacteria was high in each sample. In the range of C/N from 5 to 15, with the increase of C/N, Clostridia increased, which was the predominant classes under high C/N conditions, while the abundance of Betaproteobacteria and Actinobacteria decreased, which was low C/N system predominant classes in the condition.
The results of principal component analysis (PCA) of the inter-sample OTU are shown in Figure 6. The samples C1, C2 and C4 are grouped into one class, that is, the microbial community structure of the three samples is similar, and C3 is clustered into one class, which is different from other samples. It can be seen that C/N has a certain influence on the changes of microbial community diversity.

**Figure 6. Principal component analysis of microbial community structure**

**4. Conclusions**

With the increase of C/N within the range of 2-20, the carbon source in the system is sufficient, the photosynthesis of plants and the microbial activity is enhanced, the chlorpyrifos degrading enzyme is increased, the photosynthesis of plants is enhanced, so the removal of chlorpyrifos by the constructed wetland system, the system substrates and the concentration of chlorpyrifos in plants increased. Under the influence of C/N, the microbial community diversity of the system changed significantly. When C/N was 5, the microbial community diversity was the most abundant. Proteobacteria and Alphaproteobacteria were the predominant phylum and class.
Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 41771109) and Science and Technology Department of Jilin Province (No. 20170101074JC).

References
[1] Liang, K., Wang, Q.S., Wang, F.H., Liang, W. (2014) Research progress in the treatment of domestic sewage by constructed wetlands. J. Journal of Agro-Environment Science, 33(03): 422-428.
[2] Jia, W.L., Wu, J., Wu, A.G., Xie, H.J., Zhang, J. (2010) Effect of carbon to nitrogen ratio on the treatment effect of constructed wetland wastewater. J. Journal of Environmental Engineering, 4(04): 767-770.
[3] Zhang, Y., Zhou, Q.H., Xu, D.(2013) Denitrification effect and strengthening measures of constructed wetlands under different C/N. J. Journal of Environmental Engineering, 7(11): 4246-4250.
[4] Wang, C., Zhou, Q.H., Wu, Z.B., (2011) Research progress in organophosphorus pesticide chlorpyrifos. J. Environmental Science, 34(7): 123-127.
[5] Niu, M.F., Xu, W.D., Ming, T.S., Wang, S.Y., Wang, W., (2010) Detection Method of Chlorpyrifos from Organophosphorus Pesticide. J. Environmental Science and Technology, 33(S2): 485-487.
[6] Makino, Y., Oshita, S., Murayama, Y., Mori, M., Kawagoe, Y., & Sakai, K. (2009) Nondestructive analysis of chlorpyrifos on apple skin using uv reflectance. J. Transactions of the ASABE, 52(6): 1955-1960.
[7] Hebert, V.R., Cindy Hoonhout, A., & Miller, G.C. (2000) Use of stable tracer studies to evaluate pesticide photolysis at elevated temperatures. J. Journal of Agricultural & Food Chemistry, 48(5):1916-21.
[8] Wei, Y.L., Zhang, X.J., Li, J.S., Xu, X.H., Li, H.M., Yang, H.T. (2010) Comparison of two methods for extraction and determination of chlorpyrifos in water. J. Shandong Science,23(06):44-47.
[9] Langille, M.G.I., Zaneveld, J., Caporaso, J.G., Mcdonald, D., Knights, D., Reyes, J.A. (2013) Predictive functional profiling of microbial communities using 16srRNA marker gene sequences. J. Nature Biotechnology, 31(9): 814-821.
[10] C.,Knight, R. (2005) UniFrac: a new phylogenetic method for comparing microbial communities. J. Appl Environ Microbiol, 71: 8228-8235.
[11] Lozupone, C., Knight, R.(2005) UniFrac: a new phylogenetic method for comparing microbial communities. J. Applied and Environmental Microbiology, 71(12): 8228-8235.
[12] Xu, J.B., Wang, Y. L., Liu, M., Chen, M.J., Lin, X.G., (2018) Study on the carbon source factors of microbial metabolic activity in red soil by biolog and microcalori coupling studies. J. ActaPedologicaSinica,2018,55(01):203-212.
[13] Wu, C.Y., Chen, N., Li, Q.F., Huang, X. (2011)Research progress on chlorpyrifos-degrading bacteria and its degradation mechanism. J. Chinese Journal of Tropical Crops,32(10):1989-1994.