Optimization of Chlorpyrifos Degradation Conditions for and Microbial Community Structure Analysis in a Constructed Wetland System

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Abstract. In this study, chlorpyrifos degradation under different operating conditions was examined in a constructed wetland system, and the degradation conditions were optimized. Additionally, the microbial community structure in wetland system was analysed by high-throughput sequencing. The results are that the best chlorpyrifos degradation conditions in the constructed wetland system were pH = 8.6, sand/natural soil ratio of 1.3:1, and a planting density of 173.73/m². The actual chlorpyrifos degradation rate was 75.68%. Moreover, Proteobacteria, Bacteroidetes and Actinobacteria; Gemmatimonas, Gp6 and Parcubacteria and so on had gradually become the main chlorpyrifos degraders of dominant phylum and genera with extension in a constructed wetland system.

1. Introduction
Chlorpyrifos was the most widely used pesticide globally and had the characteristics of high efficiency and low toxicity[1-2]. With the extensive use of chlorpyrifos poisoning in agricultural production, although chlorpyrifos can protect crops, it causes damage to agricultural ecology, thus endangering human health to different degrees[3-4]. As an emerging wastewater treatment technology, the constructed wetland system has been widely applied for the prevention and control of agricultural non-point source pollution and the ecological management and restoration of rivers and lakes[5-6]. Therefore, in this study, the degradation characteristics of chlorpyrifos in a constructed wetland system under different operating conditions were studied and the degradation conditions were thus optimized. The structure and dynamic changes in the microbial community of the constructed wetland system were examined under the optimal conditions in order to provide a molecular and ecological basis for the study of system optimization and purification mechanisms.

2. Materials and methods

2.1. Experimental setup
As shown in Figure 1, the wetlands were constructed using a PVC bucket with filled to 2 cm with gravel, the upper of the wetland was filled with a mixture of sand and natural soil to form a layer of 15 cm. Meanwhile, in the wetland system Acorus calamus was planted according to different experimental conditions. During the experiment, the water level was controlled at 2 cm above the substrate.
2.2. Experimental design

Combined with the single factor experiments, based on the experimental conditions of pH = 7, sand/natural soil of 1:1, and a planting density of 171 trees/m$^2$ as the maximum response values, optimization of degradation conditions designed for the response surface experiment was conducted with 3 factors at 3 levels of the system (Table 1). The experimental wastewater was artificially simulated wastewater containing 480 g/L chlorpyrifos oil and the initial chlorpyrifos concentration was controlled at 0.48 mg/L during the experiment. The experimental period was 7 days and the experiment was repeated 5 times for each group.

Table 1. Factors and levels for response surface methodology

| Code | Factor                        | Level |
|------|-------------------------------|-------|
| A    | pH                            | -1    | 0    | 1    |
| B    | Sand/natural soil             | 1:2   | 1:1  | 2:1  |
| C    | Planting density (tree/m$^2$) | 114   | 171  | 228  |

2.3. Methods of analysis

During the experiment, water and soil samples were collected at the end of each test cycle (i.e., each 7 d), to monitor the chlorpyrifos content in water. The average value obtained from five repeated experiments (7 d each) was used for further analysis. Determination of chlorpyrifos was performed using UV spectrophotometry [7-8]. In order to analyse the structure of the microbial community and the dynamic changes, soil samples from the experimental group were collected and subjected to high-throughput sequencing under the optimal conditions. Soil samples were collected at the beginning (1$^{st}$ day), middle (14$^{th}$ day), and later period (35$^{th}$ day) of the system operation, and the specific samples were labelled as D1, D2, and D3, respectively.

3. Results and discussion

3.1. Response surface experimental results analysis

Multiple regression fitting of the experimental results was carried out with Design Expert 8.0.6 software. The regression equation (1) was formed considering the response quantity (chlorpyrifos degradation rate) and the factors (pH, sand/natural soil, planting density).

\[ Y = 72.31 + 2.15A - 0.041B + 0.16C + 0.72AB + 0.11AC + 0.39BC - 1.65A^2 - 1.70B^2 - 1.98C^2 \]  (1)
The regression analysis of the model ANOVA and the significance test results are shown in Table 2. P < 0.01 for Model indicates that the model was highly significant, had a better fitting degree, and that its response surface could be used for subsequent optimization designs. Simultaneously, the Pr > F value > 0.05 in the Lack of Fit indicated that the model was not significant, i.e. the equation had a better fitting degree to the experiment [9-11]. Moreover, the factor A in the model had a very significant effect on the chlorpyrifos degradation rate (P < 0.01). C² was significant (P < 0.05), and the other factors and their interactions had no significant effect on the chlorpyrifos degradation rate. The effect of each factor on the chlorpyrifos degradation rate was as follows: A > C > B.

Table 2. Variance analysis of response surface regression equation

| Source | Squares | df | Mean Square | F Value | Prob > F |
|--------|---------|----|-------------|---------|----------|
| Model  | 147.37  | 9  | 16.37       | 11.32   | 0.0004   |
| A      | 63.52   | 1  | 63.52       | 45.16   | 0.0001   |
| B      | 0.023   | 1  | 0.023       | 0.016   | 0.9014   |
| C      | 0.37    | 1  | 0.37        | 0.26    | 0.6236   |
| AB     | 6.43    | 1  | 6.43        | 4.45    | 0.0612   |
| AC     | 0.15    | 1  | 0.15        | 0.11    | 0.7507   |
| BC     | 1.9     | 1  | 1.9         | 1.31    | 0.2783   |
| A²     | 7.13    | 1  | 7.13        | 4.93    | 0.0507   |
| B²     | 5.78    | 1  | 5.78        | 4.00    | 0.0735   |
| C²     | 10.27   | 1  | 10.27       | 7.10    | 0.0237   |
| Residual| 14.46   | 10 | 1.45        |         |          |
| Lack of Fit | 9.79  | 5  | 1.96        | 2.1     | 0.2718   |
| Pure Error | 4.67  | 5  | 0.93        |         |          |
| Cor Total | 161.84 | 19 |              |         |          |

Note: A-pH, B-sand/natural soil, C-planting density, Prob > F values of less than 0.05 indicate significant effects on the model or the inspected factors; Prob > F values of less than 0.01 indicate very significant effects.

Using Design Expert 8.0.6 software to solve the regression equation, we determined that the best chlorpyrifos degradation conditions in the constructed wetland system were pH = 8.69, sand/natural soil of 1.36:1, and a planting density of 17.33/m². The optimal degradation rate of chlorpyrifos was predicted to be 73.05%. The optimal degradation conditions were verified and the actual measured wetland degradation rate of chlorpyrifos was 75.68%, with the maximum relative error of the predicted value and the measured value less than 5%. The optimized degradation conditions were accurate and reliable, and have practical value. The model can thus effectively predict experimental results.

3.2. High throughput sequencing analysis

As shown in Table 3, the microbial community richness (Chao1 and ACE) followed the order D3 > D2 > D1, the microbial community richness of D3 was the highest. There was no significant difference between the D1 and D2. In terms of microbial community diversity, the Shannon index was the highest for D3, whereas the Simpson index was the lowest. That is, the microbial community diversity of D3 was higher than that in other treatment groups. There was no significant difference between the Shannon index and the Simpson index of D1 and D2, which occurred in the order D3 > D2 > D1. Thus, the richness and diversity of the microbial community increased with the time of system operation is affected by chlorpyrifos.

Table 3. Analysis of the richness and diversity of microbial communities in all samples

| Treatment | Seq  | OTU  | Shannon | ACE    | Chao 1 | Simpson | Coverage |
|-----------|------|------|---------|--------|--------|---------|----------|
| D1        | 54655| 4446 | 6.08    | 6058.89| 5722.69| 0.02    | 0.97     |
| D2        | 56828| 4644 | 6.30    | 6205.79| 5890.79| 0.01    | 0.97     |
In order to determine the diversity of the microbial communities in these experiments in more detail, only the top thirteen microbial communities of each sample were analysed at the phylum level. The results are shown in Figure 2. Proteobacteria had the greatest relative abundance among the twelve microbial communities. According to the previous research, there are many organic matter and inorganic metabolism related microorganisms in Proteobacteria, and it is widely distributed in the constructed wetland system[12]. Secondly, great relative abundance of Actinobacteria, Bacteroidetes, Acidobacteria, and Firmicutes was observed. The relative abundance of the top five predominant microbial phylum in samples D1, D2, and D3 was 89.34%, 87.95%, and 65.86%, respectively. The relative abundance of Proteobacteria, Bacteroidetes and Actinobacteria was obviously the middle and late stages larger than the early stage of system operation, which indicated that the three microbial phylum were the main chlorpyrifos degraders in a constructed wetland system.

![Figure 2](image_url)  
Figure 2. Microbial community structure and relative abundance of each sample at the phylum level.

In order to further understand the dynamic changes in the microbial community structure of the system, only the top twelve microbial communities of each sample were analysed at the genera level. The results are shown in Figure 3. The predominant microbial genera in the three samples were *Sphingomonas, Massilia, Gemmatimonas, Gp6, Lysobacter*, etc. The relative abundance of five predominant microbial genera in the three samples was 16.13%, 37.95%, and 14.34%, respectively. Among the twelve predominant microbial genera, the relative abundance of *Gemmatimonas, Gp6 and Parcubacteria* was increased, this indicated the *Gemmatimonas, Gp6 and Parcubacteria* were the main chlorpyrifos degraders in a constructed wetland system at microbial genera level.
4. Conclusions

In this study, chlorpyrifos degradation under different operating conditions was examined in a constructed wetland system, and the degradation conditions were optimized. The microbial community structure in wetland systems was also analysed by high-throughput sequencing.

1) Combined with the response surface method, the best chlorpyrifos degradation conditions in the constructed wetland system were pH = 8.69, sand/natural soil of 1.36:1, and planting density 17.33/m². The optimal chlorpyrifos degradation rate was predicted to be 73.05%. The actual rate was found to be 75.68%. No significant difference was observed between the predicted and actual value. Thus, the model could effectively predict the experimental results.

2) High-throughput sequencing showed that the number of microbes was increased with the extension of operating time of the constructed wetland system where Proteobacteria, Bacteroidetes and Actinobacteria; Gemmatimonas, Gp6 and Parcubacteria and so on had gradually become the main chlorpyrifos degraders of dominant phylum and genera in a constructed wetland system.

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References

[1] Agudelo R M., Peñuela G., Aguirre N J., et al. (2010) Simultaneous removal of chlorpyrifos and dissolved organic carbon using horizontal sub-surface flow pilot wetlands. Ecological Engineering. 36(10): 1401-1408.
[2] Karpuzcu M E., Sedlak D L., Stringfellow W T. (2013) Biotransformation of chlorpyrifos in riparian wetlands in agricultural watersheds: implications for wetland management. Journal of Hazardous Materials, 244-245(2):111-120.
[3] Lazic’ S., Šunjka, D., Grahovac N., et al. (2012) Determination of chlorpyrifos in water used for agricultural production. Agriculture & Forestry, 17-25.
[4] Rogers M R., Stringfellow W T. (2009) Partitioning of chlorpyrifos to soil and plants in vegetated agricultural drainage ditches. Chemosphere, 75(1):109-114.
[5] Stefanakis A I., Seeger E., Dorer C., Sinke, A., Thullner, M., (2016) Performance of pilot-scale horizontal subsurface flow constructed wetlands treating groundwater contaminated with phenols and petroleum derivatives. Ecol. Eng. 95,514-526.
[6] Vymazal J., Greenway M., Tonderski K., et al. (2005) Constructed Wetlands for Wastewater Treatment. C R C Critical Reviews in Environmental Control, 25(5):475-477.

[7] Niu, M.F., Xu, W.D., Ming, T.S., et al. (2010). Organophosphorus pesticide chlorpyrifos detection. Environ. Sci. Technol. (s2), 485-487.

[8] Makino Y., Oshita S., Murayama Y., et al. (2009) Nondestructive Analysis of Chlorpyrifos on Apple Skin Using UV Reflectance. Transactions of the Asabe, 52(52):1955-1960.

[9] Bezerra M A., Santelli R E., Oliveira E P., et al. (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta, 76(5):965-977.

[10] Myers R H., Montgomery D C. (2008) Response Surface Methodology: Process and Product in Optimization Using Designed Experiments. Technometrics, 38(3):284-286.

[11] Zhou J., Li H., Chen X., et al. (2017) Cometabolic degradation of low-strength coking wastewater and the bacterial community revealed by high-throughput sequencing. Bioresource Technology, 245: 379-385.

[12] Ansola, G., Arroyo, P., Le, S.D.M., (2014) Characterisation of the soil bacterial community structure and composition of natural and constructed wetlands. Total Environ. s473–474(3), 63-71.