

Effect of Rhizosphere on Sediment Microbial Numbers in Phytoremediation Process of Decabromodiphenyl Ether Contaminated Sediment

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Abstract. In order to investigate the rhizosphere effect caused by the aquatic macrophyte *Scirpus validus* during the phytoremediation process of decabromodiphenyl ether contaminated sediment, the sediment microbial number changes in three typical sediment rhizosphere, including bacteria, fungi and actinomycetes were examined. The results showed that the number of microorganisms in sediments was significantly increased by planting and the number of bacteria and fungi increased by 2-12 times and 1-4 times in the respective rhizosphere sediment. No obvious difference of quantity of actinomycetes was observed between rhizosphere and non-rhizosphere in silt and clay sediments expect for sand sediment. The results suggested that the numbers of microorganisms can be stimulated by the presence of *S. validus* and the rhizosphere effect was obvious.

1. Introduction

Rhizosphere is the micro region environment, influenced by plant roots and its activities in soil or sediments[1]. The rhizosphere environment is a few millimeters area around the plant root, which is different from the original soil environment due to its special physical, chemical and biological characteristics[2]. At present, a number of research have been demonstrated that microbial degradation is one of the major mechanisms for the phytoremediation of organic pollutants contaminated soil. Microbes, especially plant rhizosphere microbes, play a crucial role in the remediation process of organic contaminated soils.

In plant rhizosphere micro-ecosystems, the well-developed root system of plants provides an environment for the microorganism, transports nutrients and oxygen to the roots, promotes the growth and metabolism capability of the rhizosphere microorganisms, thus enhance the biological transformation capacity of microorganisms [3]. Root exudates such as carbohydrates, organic acids, phenols, amino acids and other organic matter can be used as C source and N source for the growth of pollutant-degrading bacteria and maintain long-term survival of bacteria[4-5]. Therefore, the rhizosphere environment possess a high level of microbial activity, diversity and biomass, which plays an important role in increasing the degradation rate of pollutants in soil. The ability of rhizosphere microorganisms to degrade many organic pollutants is well-known.

In this study, the changes of microbial numbers in rhizosphere and non-rhizosphere sediments of aquatic macrophyte *Scirpus validus* during the phytoremediation of sediment contaminated with BDE-



209 were analyzed. The dynamic characteristics of rhizosphere microorganisms and the rhizosphere effect caused by microorganisms in the process of phytoremediation can provide some theoretical basis for revealing the mechanism involved in phytoremediation of decabromodiphenyl ether (BDE-209) contaminated sediment.

2. Materials and methods

2.1. Chemicals and plants

Decabromodiphenyl ether (BDE-209, $C_{12}OBr_{10}$) was purchased from *Alfa Aesar* (Johnson Matthey, USA) with 99% chemical purity (GC). Standard of BDE-209 was purchased from Sigma-Aldrich (Sigma-Aldrich, Inc., St. Louis, MO).

S. validus used in the experiment were collected from the Tangxun Lake in Wuhan, Hubei Province, China. Plants with initial height of 30-50 cm were cultured in the artificial pond for 10 days before experiments.

2.2. Experimental set-up

Three typical types of sediments (silt, clay, and sand) were collected from the 0-20 cm surface sediments at a local lake in Wuhan, Hubei Province, China. All collected sediments were then air-dried, and passed through a 2-mm sieve. BDE-209 contaminated sediments were prepared by the sprinkling method[6-7], and finally BDE-209 concentration was approximately 2 mg kg^{-1} .

For the experiment, 15 kg of contaminated dry sediments (approximately 2 mg kg^{-1}) were loosely packed into cultivation box, each treatment had triplicates and 20 individuals of *S. validus* with initial plant height of about 40 cm were transplanted into each box. Tap water was then added and kept at the level 4 cm above the sediment surface. The experiment was carried out in natural conditions. Three treatments were established for each type of sediment as follows: original sediments; sediments with BDE-209 at 2 mg kg^{-1} ; sediments planted with *S. validus*; sediments with BDE-209 at 2 mg kg^{-1} and *S. validus*.

2.3. Analysis of bacterial in sediment

The bacterial abundance of sediment samples were enumerated by the 4', 6'-diamidino-2-phenylindole (DAPI) direct count method and cells counted on an epifluorescence microscope (Laphot, Nikon, Japan).

2.4. Analysis of fungus in sediment

1.0 g of fresh sediment of each sample was diluted with sterile water to prepare a dilution of 10^{-1} , 10^{-2} , 10^{-3} sediment concentration. 200 μL of sediment solution was inoculated in culture dish with Martin-Bangladesh red medium. Three replicates were set for each concentration gradient and incubated at constant temperature (28°C) for 3-4 days. The number of microorganisms is calculated as \log^{10} of the number of fungi per gram of sediment (dw). The cultivation substrate components for fungi is displayed in table 1.

Table 1. The cultivation substrate components for fungi

Ingredient	Dosage
Glucose	10.0 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5g
KH_2PO_4	1.0 g
Agar	18. 0 g
Peptone	5.0 g
Rose Bangal	1%, 3.3mL
Streptomycin (1%)	3 mL
Distilled water	1000 mL

2.5. Analysis of actinomycetes in sediment

1.0 g of fresh sediment of each sample was diluted with sterile water to prepare a dilution of 10^{-1} , 10^{-2} , 10^{-3} sediment concentration. 200 μ L of sediment solution was inoculated in culture dish with actinomycetes culture medium. Three replicates were set for each concentration gradient and incubated at constant temperature (28°C) for 7-10 days. The number of microorganisms is calculated as \log^{10} of the number of fungi per gram of sediment (dw). The cultivation substrate components for actinomycetes is displayed in table 2.

Table 2. The cultivation substrate components for actinomycetes

Ingredient	Dosage
KNO ₃	1.0g
FeSO ₄ ·7H ₂ O	0.01g
K ₂ HPO ₄	0.5g
MgSO ₄ ·7H ₂ O	0.5 g
NaCl	0.5g
K ₂ Cr ₂ O ₇	0.01g
Agar	18. g
Starch	20g
Distilled water	1000 mL
pH	7.2-7.4

3. Results and discussions

The changes of bacteria, fungi, and actinomycete in the rhizosphere and non-rhizospheric of three typical sediments during the phytoremediation process are shown in table 3. The microbial numbers in the silt, clay and sand sediments planted with *S. validus* were significantly higher than those in sediment control in the phytoremediation except for actinomycete. The bacteria and fungi numbers of rhizosphere fractions in silt, clay and sand sediments were almost 2-12 times and 1-4 times more than the corresponding sediment controls, respectively. The above results clearly demonstrated that the microbial populations were significantly enhanced by growth of *S. validus*, the rhizosphere effect is obvious. For actinomycete, although the number of actinomycetes in the initial rhizosphere sediment was higher than that in the non-rhizosphere in the silt and clay experimental group, the number of actinomycete did not increase significantly at the late stage. However, for sandy sediment, the number of actinomycetes in the rhizosphere sediments was higher than that in the whole phytoremediation and 97.73%~215.79% of actinomycetes were increased, indicating that the planting of *S. validus* can greatly promote the amount of actinomycetes in sand sediments. The above results show that the planting of aquatic macrophyte *S. validus* can greatly increase the number of actinomycetes in three typical sediments, and its rhizosphere effect is obvious.

Rhizosphere is biologically active soil region where macrophyte roots interact with sediment and microbes and roots provide suitable habitats for the growth of the microorganisms[8]. Furthermore, plant exudation and root sloughing can also affect the activities of microbes and changes in exudates released resulting in associated effects on biodegradation and microbial populations[9-10]. The result showed that the microbial populations were significantly increased upto 5-12 times as those in unplanted controls in the rhizosphere sediments, indicating that the growth of microorganisms was stimulated by the presence of *S. validus*.

Table 3. Changes of bacterial amounts in three typical sediments

Sediment	Treatments	Remediation time (d)					
		30	60	90	120	150	180
Silt	Silt	8.702±0.157	8.818±0.048	8.887±0.055	8.87±0.091	8.765±0.096	8.686±0.150
	Silt+BDE-209	8.793±0.022	8.857±0.076	8.920±0.043	8.906±0.0132	8.804±0.036	8.688±0.086
	Silt+ <i>S. validus</i> Vahl	9.315±0.134	9.598±0.160	9.715±0.082	9.622±0.048	9.655±0.147	9.416±0.122
	Silt+BDE-209+ <i>S. validus</i> Vahl	9.372±0.121	9.683±0.115	9.638±0.059	9.685±0.041	9.719±0.098	9.460±0.151
Clay	Clay	8.322±0.318	8.472±0.170	8.507±0.235	8.550±0.120	8.454±0.278	8.406±0.203
	Clay+BDE-209	8.551±0.278	8.578±0.298	8.524±0.282	8.565±0.186	8.509±0.249	8.410±0.283
	Clay+ <i>S. validus</i> Vahl	9.397±0.085	9.590±0.148	9.550±0.130	9.504±0.131	9.456±0.199	9.390±0.068
	Clay+BDE-209+ <i>S. validus</i> Vahl	9.294±0.156	9.472±0.159	9.519±0.062	9.405±0.165	9.518±0.050	9.322±0.186
Sand	Sand	7.449±0.087	7.615±0.266	7.744±0.182	7.746±0.150	7.715±0.112	7.636±0.236
	Sand+BDE-209	7.706±0.085	7.754±0.179	7.753±0.257	7.799±0.208	7.724±0.152	7.667±0.217
	Sand+ <i>S. validus</i> Vahl	8.407±0.077	8.657±0.075	8.869±0.044	8.812±0.065	8.808±0.082	8.702±0.022
	Sand+BDE-209+ <i>S. validus</i> Vahl	8.506±0.115	8.722±0.170	8.902±0.123	8.848±0.179	8.755±0.163	8.727±0.142

Table 4. Changes of actinomycetic amounts in three typical sediments

Sediment	Treatments	Remediation time (d)					
		30	60	90	120	150	180
Silt	Silt	4.272±0.124	4.508±0.119	4.793±0.118	4.753±0.193	4.865±0.084	4.742±0.097
	Silt+BDE-209	4.339±0.179	4.638±0.268	4.902±0.131	4.929±0.089	4.959±0.109	4.592±0.109
	Silt+ <i>S. validus</i> Vahl	4.730±0.140	4.997±0.093	5.036±0.0.136	5.989±0.107	5.053±0.182	4.941±0.102
	Silt+BDE-209+ <i>S. validus</i> Vahl	4.668±0.083	4.923±0.229	4.944±0.104	5.903±0.142	4.763±0.167	4.710±0.134
Clay	Clay	4.455±0.168	4.719±0.127	5.016±0.114	4.938±0.168	4.841±0.177	4.571±0.110
	Clay+BDE-209	4.644±0.144	4.893±0.127	5.016±0.114	4.938±0.168	4.841±0.177	4.571±0.110
	Clay+ <i>S. validus</i> Vahl	4.973±0.145	5.102±0.080	5.107±0.109	5.105±0.066	5.094±0.011	4.903±0.127
	Clay+BDE-209+ <i>S. validus</i> Vahl	4.973±0.217	5.153±0.037	5.320±0.026	5.337±0.027	5.322±0.067	5.037±0.137
Sand	Sand	3.708±0.369	4.006±0.138	4.111±0.088	4.058±0.094	4.094±0.108	3.871±0.152
	Sand+BDE-209	3.834±0.191	4.095±0.064	4.215±0.043	4.184±0.151	4.159±0.093	3.962±0.166
	Sand+ <i>S. validus</i> Vahl	4.145±0.103	4.422±0.127	4.519±0.106	4.489±0.151	5.453±0.111	4.423±0.097
	Sand+BDE-209+ <i>S. validus</i> Vahl	4.191±0.073	4.487±0.128	4.541±0.119	4.473±0.138	4.471±0.063	4.468±0.125

Table 5. Changes of fungi amounts in three typical sediments

Sediment	Treatments	Remediation time (d)					
		30	60	90	120	150	180
Silt	Silt	3.875±0.124	4.138±0.131	4.245±0.095	4.087±0.093	4.043±0.069	4.000±0.091
	Silt+BDE-209	3.802±0.247	4.081±0.080	4.153±0.091	4.004±0.132	4.011±0.075	3.911±0.090
	Silt+ <i>S. validus</i> Vahl	4.035±0.073	4.323±0.032	4.363±0.052	4.331±0.103	4.150±0.148	4.211±0.211
	Silt+BDE-209+ <i>S. validus</i> Vahl	4.081±0.062	4.281±0.056	4.387±0.053	4.277±0.065	4.232±0.086	4.181±0.168
Clay	Clay	3.413±0.194	3.644±0.158	3.758±0.048	3.582±0.128	3.548±0.061	3.506±0.105
	Clay+BDE-209	3.426±0.176	3.731±0.108	3.703±0.085	3.524±0.046	3.453±0.054	3.355±0.108
	Clay+ <i>S. validus</i> Vahl	3.758±0.141	3.937±0.113	4.080±0.078	4.037±0.084	4.018±0.111	3.738±0.177
	Clay+BDE-209+ <i>S. validus</i> Vahl	3.819±0.095	3.968±0.157	4.028±0.078	4.091±0.042	3.972±0.046	3.706±0.132
Sand	Sand	2.967±0.164	3.312±0.141	3.608±0.063	3.478±0.075	3.372±0.277	3.230±0.213
	Sand+BDE-209	3.026±0.081	3.411±0.188	3.661±0.136	3.393±0.195	3.392±0.1160	3.326±0.172
	Sand+ <i>S. validus</i> Vahl	3.710±0.208	3.815±0.140	3.867±0.157	3.805±0.091	3.798±0.164	3.548±0.213
	Sand+BDE-209+ <i>S. validus</i> Vahl	3.804±0.199	3.922±135	3.977±0.160	3.923±0.077	3.873±0.056	3.622±0.110

4. Conclusion

The rhizosphere effect caused by the aquatic macrophyte *Scirpus validus* during the phytoremediation process of decabromodiphenyl ether contaminated sediment was investigated, the sediment microbial number in three typical sediment rhizosphere, including bacteria, fungi were significantly increased due to the rhizosphere. No obvious difference of quantity of actinomycetes was observed between rhizosphere and non-rhizosphere in silt and clay sediments except for sand sediment. The results suggested that the numbers of microorganisms can be stimulated by the presence of *S. validus* and the rhizosphere effect was obvious. The results can provide data of theoretical basis for revealing the mechanism involved in phytoremediation of decabromodiphenyl ether (BDE-209) contaminated sediment.

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