Performance and Mechanism of the in-situ Restoration Effect on VHCs in the Polluted River Water Based on the Orthogonal Experiment: Photosynthetic Fluorescence Characteristics and Microbial Community Analysis.

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Performance and mechanism of the in-situ restoration effect on VHCs in the polluted river water based on the orthogonal experiment: photosynthetic fluorescence characteristics and microbial community analysis.

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Abstract:

Volatile halogenated hydrocarbons (VHCs) attracted many attentions due to its toxic and persistent in the environment. In this research, a novel in situ ecological restoration reactor was applied to the degradation of VHCs in polluted river water. The optimized working condition adaptation of the in-situ restoration technique was evaluated through orthogonal tests. The experiments showed that when the water depth was 0.4 m, the HRT was 5d and the current velocity was 1 m/s, VHCs removal efficiencies could achieve favorable value. The $CHCl_3$ CCl_4, C_2HCl_3 and C_2Cl_4 removal efficiency could reach 70.27%, 70.59%67.74% and 81.82%, respectively. F test results showed that both HRT and water depth were significantly related to the removal efficiency of reactor. Besides, using underwater modulated chlorophyll fluorometer analyzed the rapid light curves (RLC) of plants in the experiment, which showed that the VHCs of damaged river was harmful to the physiological state of the plants. Moreover, the microbial community structures of fillers in the reactor were tested by high-throughput sequencing, the findings supported that the microbial community made a great response to adapt to the changes of environment of the reactor. The relative abundance of *Rhodocyclaceae* increased slightly, which hinted that it had good adaptability to VHCs in polluted river water. The research results confirmed that in situ ecological restoration reactor was an economical technology for removal VHCs in polluted river water.

Key words: Volatile halogenated hydrocarbons; Orthogonal tests; in-situ ecological restoration; Microbial community

1.Introduction

Urbanization in China has accelerated the transformation of geomorphological features and ecological environment in urban river, which contained the decrease on the hydrological connectivity and the pollutants accumulated at the bottom of the river channels (Wang et al., 2017). With the process of urbanization continues to move forward, the destruction intensified of urban water environment, especially in urban rivers and lakes environment (Lu et al., 2018). Human activity changes the original shape of the river, injects additional pollutants to affect the water quality frequently (Hirsch et al., 2010). Volatile halogenated hydrocarbons (VHCs) were constituted with a series of diversified organic compounds, most of which with the characteristics of environmental persistence, bioaccumulation and biology toxicity (Zou et al., 2006; Song et al., 2017). Meanwhile, some VHCs instant vaporized into air environment as a consequence of low boiling points, adding air pollutant and the local people risk of illness in urban area (Mun & Townley, 2021). VHCs has been widely accepted as a major source of photochemical smog since the beginning of the 21th century. Besides, variety of VHCs produced by plastics petroleum and landfill leachate influx into urban river alteration the characteristics of water environment and initiation potential adverse effects on human (Wang et al., 2020). VHCs would cause continuous hazard in environmental media, which was the particularly sensitive and prominent in river within the city limits.

River pollution caused by human activities is very common in world, many regions and countries begin to show concerns for the river quality and take measures to treat it. As we all know, many factors such as the water depth and current velocity, regulate actual situation of the river and affect pollutant distribution in the damaged river (Jiang et al., 2017; Rugner et al., 2019). Due to the complexity characteristics of river pollution, the traditional water treatment process is difficult for practical application (Giripunje et al., 2015).

Actually, both phytoremediation and slope wetland system are known as green and efficient in situ water environment remediation treatments, which could alter water quality and flow regime to a certain extent (Ladislas et al., 2011). *Submerged macrophytes* as a representative eco-engineering plants, it also is an important component of river ecosystem, which have capable of influencing various biotic and abiotic environments, including the status of the rivers (Zhang et al., 2018; Özgencil et al., 2020). Slope wetland system has characterized by good geomorphology adaptability and flexible controllability, which was suitable for recovery and remediation of damaged river (Wang et al., 2021). Aimed at maintenance and restoration polluted river water, the two technologies need to be suitable combination with low cost and simple management mode (Nilsson et al., 2015). The combination of the two methods can not only expand the suitable construct methods to the riverway physiognomy, but also improve the removal efficiency on the organic matters in the polluted river water. Additionally, both *Submerged macrophytes* and slope wetland are biological systems involved in the degradation of organic matter. And the plants in conjunction with microflora in the system, which would play cleaning function on the coupling system by the bioremediation function (Wang et al., 2017). Hence, the regulation on plant physiological activities and microbial community status was a vital concern, which involve in optimized the pollutant removal and performance on coupling system.

Water pollution prevention action plan has been applied to numerous black-stinking water bodies in China, which met the requirements on elimination eutrophication and promote water resources management (Song et al., 2017). Many rivers restoration projects have been applied to urban river remediation through the last few years, but the degradation VHCs in polluted river water have been rarely reported. In view of VHCs are toxic and persistent in the environment, determining its content of polluted river water and proposed appropriate measures to restore the river ecosystem is essential measures to local population health.

In view of the above aspects, in situ restoration reactor was used as the research carrier, and the purification effect on VHCs in the polluted river water was investigated. After due consideration, different experimental factors were set on orthogonal the outdoor simulation experiment to determine the optimal conditions on the reactor. The underwater modulation chlorophyll fluorescence was employed to analyze the physiological state of plants at the in-situ restoration reactor at the same. In addition, the headspace gas chromatography was used as detected the content of VHCs in damaged river and tested the VHCs variation on polluted river water under the system treatment. Besides, the change of substrate microorganism was measured before and after the experiment to evaluate the flora on the dichlorination reaction. The aim of this research was to explore and optimize the actual operation requirement of the in-situ restoration reactor through microbial resource management and some relevant analysis instruments. And the impact on experimental factors were evaluated and pollutant decomposition mechanism in the reactor were comprehensively analyzed.

2. Materials and methods

2.1 Experimental device

To assure an effective and authenticity research, the experiment was carried out outdoors. One device was set up in the experiment, which dimension was 1m high, 2m wide and 7m long. The experimental devices were consisted of water inlet pipe, water outlet, water tank and sampling ports. In the flank of all reactors, three wastewater sampling pipes are arranged in three rows with the height of 0.4m, 0.6m and 0.8m (to the bottom). Slope wetland contained three plants (*ScirpusvalidusVahl, Typha orientalis Presl* and *Lythrum salicaria L.*), which was planted from bottle to up, respectively. And planting density was set at 30clumps/m^2. *Potamogeton wrightii Morong* and *Potamogeton pectinatus* was closely adjacent seeded around the *ScirpusvalidusVahl* in the sediment of the reactor, and the planting density was set at 30clumps/m^2. The pushing flow device (QJB0.85) was purchased from Sapphire environmental protection (Nanjing, China), and impeller diameter was 220mm. In order to guarantee the replenishment and drain of the reactor steadily, the polluted river water employed in the reactor was supplied through the water tank. In addition, the polluted river water directly extracted from the damaged river by the water pump, which enhance the reliability of the simulation experiment and guarantee the authenticity of the results to a certain extent. The experimental reactor is shown in Fig.1.

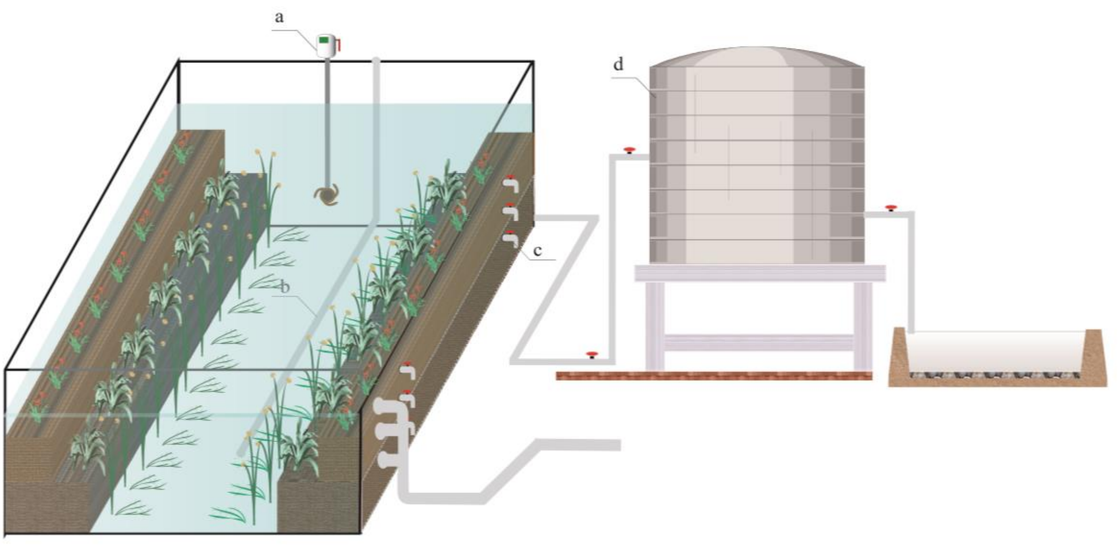


Fig.1 Experimental reactor (a. plug flow device b. water replenishing pipe c. sampling ports d. water tank)

2.2 Experimental chemical and instrument

The chemicals obtained methanol and ethanol, which was used in the experiment were of guaranteed reagent (GR) and purchased from Beijing chemical plant (Beihua, China). Standard solutions (GSB07-1982-2005) of 1,000 μg·mL^{-1} VHCs in ethanol were purchased from standard sample Institute of Ministry of environmental protection, including $CHCl_3$, CCl_4, C_2HCl_3 and C_2Cl_4. Analytical grade of NaCl were purchased from Yongda Chemical Reagent Co., Ltd (Tianjin, China). Headspace bottle and micropipettor (1 μL, 10 μL, 50 μL) were purchased from high pigeon instrument (Shanghai, China). 1,000 μg·mL^{-1} chloride standard solutions (GSB 07-1267-2000) was purchased from the resources platform of the national standard material. Main instruments of the experiment contained: headspace sampler (7697A, Aglient, America), headspace gas chromatograph (7890A, Aglient, America), capillary chromatographic column (HP-5MS, Aglient, America), ultra-pure water machine (CM-R0-C2, Anshi, China), underwater modulated chlorophyll fluorometer (DIVING-PAM-II, Zealqueit, China) and associated accessories.

2.3 Gas chromatography operating conditions

HS-GC analysis conducted with the environmental protection standards of HJ620-2011. The micropipette was used as transfer standard solutions of VHCs, which was diluted by methanol to obtain the 10 μg·mL^{-1}preparation solutions. Preparation solutions was allowed the headspace extraction and gas phase operations relatively handy. The NaCl was dried to a constant weight (350℃ for 3h) and then cooled at room temperature, which was added into headspace bottle followed immediately when it has cooled. At the same time, 5, 10, 20, 30, 50μL preparation solutions was added up respectively into 10mL ultra-pure water in the headspace bottle, and the mixture sufficient agitated respectively. After the above operations, the standard solution was obtained. HS-GC operations were composed of headspace injection, high temperature separation and analyte display. A robotic arm and a headspace generation unit was consisted the 111-space autosampler, which could heat samples and convey the bottles into the GC equipment. Chromatographic conditions: Initial temperature was 40℃ for 2 min and increased to 100 ℃ at 60 ℃ min^{-1}, then 100 ℃ to 200 ℃ at 10 ℃ min^{-1}. Detection temperature was set as 320 ℃. Helium used as carrier gas and flow velocity was 1.0mL·min^{-1}. The results were shown in the Fig.2a).

The signal was gathered from GC equipment, which reflected the characteristic on analytes by the position and size of peak area. The external standard method was used to establish standard line, the results were shown in the Fig.2b).

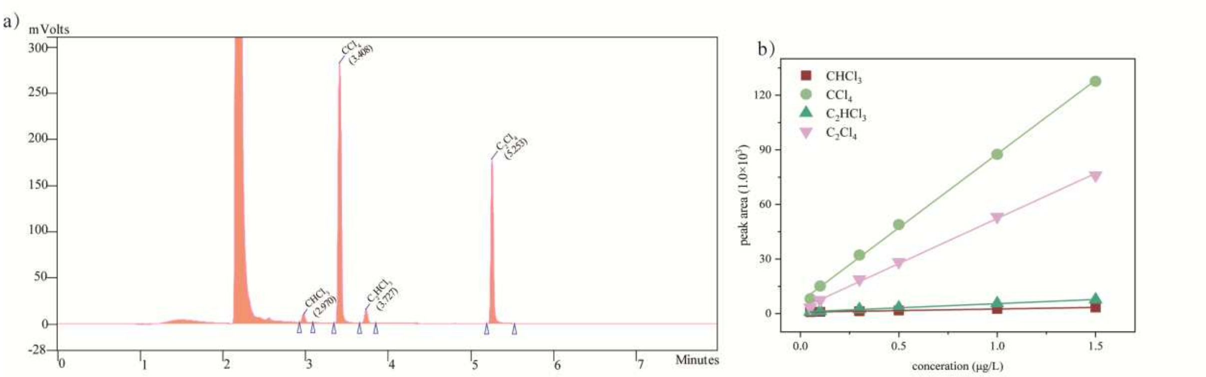


Fig.2 Gas chromatogram analysis of target compounds (a. Gas chromatogram of VHCs b. Standard line of VHCs)

2.4 Experimental site and influent quality

In this study, the Xiaozhong river in Tongzhou District of Beijing was selected as experimental site. The average annual atmospheric temperature of Tongzhou District is 13-24°C. The river runs through an urban area and it adopts the way of reclaimed water replenishment, and the main stream is set with several sewage outlets, the self-purification capacity of water body is poor. The experimental site is located in Tongzhou Xiaozhong River experimental base (116°67'E, 39°93'N), and the sampling point is located at the confluence point of five rivers. Its variation can effectively reflect the interaction between upstream and downstream water quality. The experimental site is shown in Fig.3.

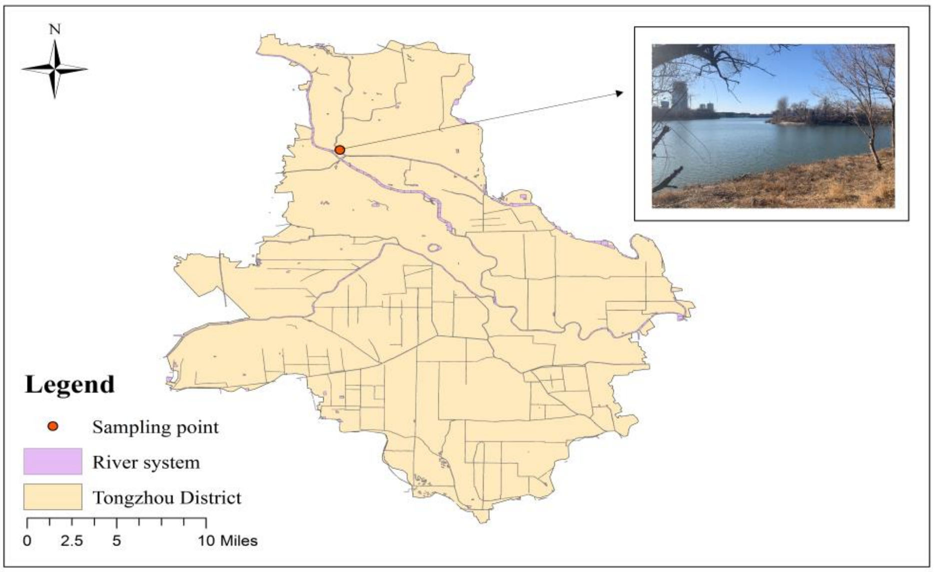


Fig.3 Experimental site

The Xiaozhong river receives multiplex sewage throughout the year, the pollutants mainly come from the surrounding domestic garbage, sewage treatment plants and rainfall, which aggravated river channel damaged situation (Zhang et al., 2020a). The integral state of river was sluggish, and some area existed stagnant zone. Using the 1000mL sample bottle, water samples were directly collected from the experimental site and were analyzed in the laboratory (sampling two times). The characteristics of polluted river water are shown in Tab. 1

Table 1 Characteristics of polluted river water

Parameter	$CHCl_3$(μg•L^{-1})	CCl_4(μg•L^{-1})	C_2HCl_3(μg•L^{-1})	C_2Cl_4(μg•L^{-1})	pH
Content	0.21	0.17	0.37	0.11	5.9~7.1

2.5 Experimental methods

In this study, in-situ restoration effect on polluted river water were evaluated by orthogonal tests under the water depth, HRT and current velocity as variables. In order to simulate the influence on river flow, considering the characteristics of wetlands at the same, the variation on HRT was applied to the reactor to meet the requirements. The HRT was calculated by the Eq.1. The three factors were selected as influencing factors, containing HRT(1, 3, and 5d), current velocity(1, 2, and 3m/s) and water depth(0.4, 0.6, and 0.8m) and three levels were taken for each factor. And the fictitious factors were added to the orthogonal matrix, for avoiding the influence of error caused by stochastic factors.

$$HRT = \frac{V}{Q} \tag{1}$$

V—The treatment volume in reactor (m^3)

Q—The influent flow in reactor (m^3/d)

Meanwhile, the methods of outdoor simulation experiment were adopted to the experiment to assure the factuality of results. DNA was extracted from the soil and filler samples with a FastDNA skin for soil Isolation kit (116560200, MP, America) on the basis of product description. The extracted DNA was stored at -20°C and follow operation contain amplification, enrich and construct the template of miseq library was finished by Beijing Allwegene Tech and Monitoring Technology Co., Ltd.. The microbial community analysis of the experiment was completed by illuminamiseq pe300 sequencing platform. Water samples were collected in glass sampling bottle (50mL) and stored at 4°C until detect. The samples before and after treatment conveyed by using headspace sampler (HS) and the content of VHCs in water samples was measured by headspace gas chromatograph (GC) with the help of capillary chromatographic column. Plants physiological status were analyzed by underwater modulated chlorophyll fluorometer.

3. Results and discussion

3.1. The degradation on VHCs under orthogonal experiment

3.1.1. Construction of orthogonal experiment

Transport of pollutants in the damaged river are closely associated with hydrodynamics situation on the polluted river water, which included current velocity, river flow, water depth and the like (Anwar Sadat et al., 2020). $L_9(3^4)$ orthogonal experiment was applied in this study. Details of the factors and levels in orthogonal experiment is shown in Tab. 2.

Table 2 Factors and levels in the orthogonal experiment

Factors	Level		
	1	2	3
Water depth (m)	0.4	0.6	0.8
HRT (d)	1	3	5
Current velocity (m/s)	1	2	3
Fictitious factors	1	2	3

3.1.2 Orthogonal experiment results

Aiming to experimental materials decrement, orthogonal experiments were constructed on representative points with mutual independence, which could validate the experimental result under various factors and obtained the optimization designation (Wang et al., 2019). The removal efficiency under different experimental variables were analyzed.

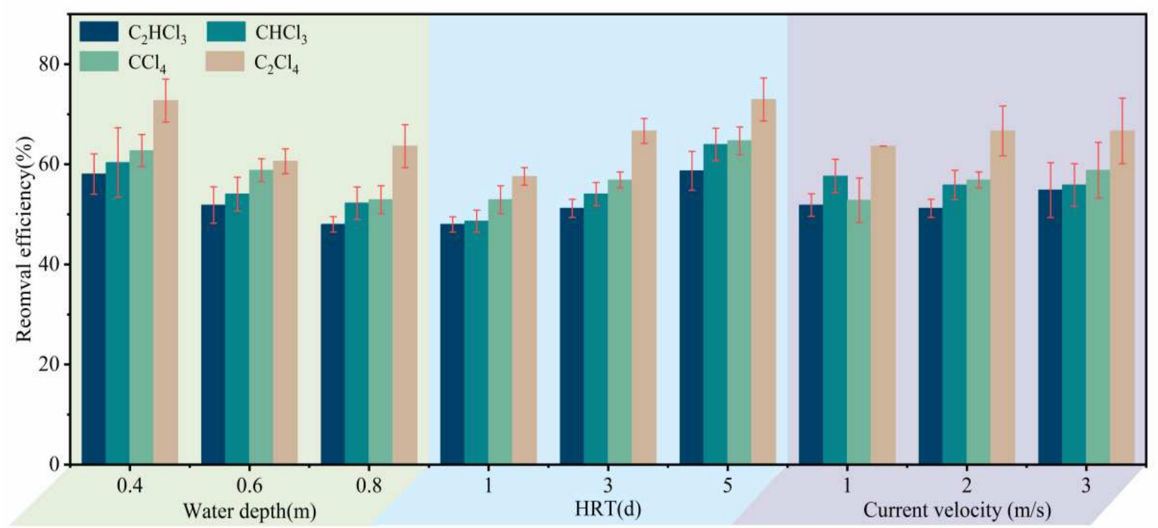


Fig.4 The VHCs removal efficiency under different influencing factors

As is shown in the Fig.4, the removal efficiency of four VHCs existed differences, which was associated with the change of influencing factors. The average removal efficiency of $CHCl_3$, CCl_4, C_2HCl_3 and C_2Cl_4 in the in-situ restoration reactor was 45.95%–70.27%, 47.06%–70.59%, 45.16%–67.74%, and 54.55%–81.82%, respectively. For the sake of determine the effect on experimental factors, significance tests of orthogonal experiment results were analyzed. The results were shown in the Table.3.

Table 3 Orthogonal matrix and significance test of results

Number of groups	Water depth (m)	HRT (d)	Current velocity (m/s)	Fictitious factors
1	0.4	1	1	1
2	0.4	3	2	2
3	0.4	5	3	3
4	0.6	1	2	3
5	0.6	3	3	1
6	0.6	5	1	2
7	0.8	1	3	2
8	0.8	3	1	3
9	0.8	5	2	1

	Name	$CHCl_3$	CCl_4	C_2HCl_3	C_2Cl_4
Water depth(m)	K_1	181.08	188.23	174.19	218.19
	K_2	162.16	176.47	155.59	181.83
	K_3	156.76	158.82	143.99	190.92
	$\bar{K}_1$	60.36	62.74	58.06	72.73
	$\bar{K}_2$	54.05	58.82	51.86	60.61
	$\bar{K}_3$	52.25	52.94	48	63.64
	R	24.32	29.41	30.2	36.36

HRT (d)	K_1	145.95	158.82	143.99	172.74
	K_2	162.16	170.58	153.67	200.01
	K_3	191.89	194.12	176.11	218.9
	$\bar{K}_1$	48.65	52.94	48	57.58
	$\bar{K}_2$	54.05	56.86	51.22	66.67
	$\bar{K}_3$	63.96	64.71	58.7	72.97
	R	45.94	35.3	32.12	46.16
Current velocity (m/s)	K_1	172.97	176.47	155.59	190.92
	K_2	167.57	170.58	153.67	200.01
	K_3	167.57	176.47	164.51	200.01
	$\bar{K}_1$	57.66	52.82	51.86	63.64
	$\bar{K}_2$	55.86	56.86	51.22	66.67
	$\bar{K}_3$	55.86	58.82	54.84	66.67
	R	5.4	5.89	10.84	9.09
Fictitious factors	K_1	162.16	176.46	154.83	200.01
	K_2	167.57	170.59	156.76	190.92
	K_3	170.27	176.47	162.18	200.01
	$\bar{K}_1$	54.05	58.82	51.61	66.67
	$\bar{K}_2$	55.86	56.86	52.25	63.64
	$\bar{K}_3$	56.76	58.82	54.06	66.67
	R	8.11	5.88	7.35	9.09

Sum of square deviance are important index for description the degree of dispersion, which was calculated by Eq.(2) and Eq.(3) in this experiment.

$$SS_t = \frac{1}{r}\sum_{i=1}^{k} K_{ij}^{\ 2} - T \quad (2)$$

$$T = \frac{S^2}{n} \quad (3)$$

SS_t—Sum of square deviance with each factors

r,k—The repeat times of each level, the number of levels with each factor

K_{ij}—Total indicators of each factor level

T—Sum of all index

n—Number of experiments (n=9)

$$N = df_a = df_b = df_c = df_d = m - 1 \quad (4)$$

$$MS_t = \frac{SS_t}{df_t} \quad (5)$$

N—Degree of freedom

MS_t—Variance of each factor

According to Eq.(4) and Eq.(5) calculated the variance of each factor. Final F value was obtained through Eq. (6) and identify the significance on the factors.

$$F_t = \frac{MS_t}{MS_e} \tag{6}$$

Using the forgoing formulas carried out the experiment data analysis of significance test. The critical value $F_{0.05}(2,4)$ of 19.247 was selected. The results were list in Tab. 4.

Table 4 ANOVA of the orthogonal experimental results

Source of variation		SS	DOF	MS	F	F_{c1}	F_{c2}	Sig
$CHCl_3$	Water depth	108.732	2	457.45	80.466	19.247	9.243	**
	HRT	361.902	2	180.951	31.83	19.247	9.243	**
	Current velocity	914.899	2	54.336	9.558	19.247	9.243	*
	Fictitious factors	11.37	2	5.685				
CCl_4	Water depth	146.085	2	73.046	18.948	19.247	9.243	*
	HRT	215.391	2	107.696	28.082	19.247	9.243	**
	Current velocity	7.709	2	3.855	1	19.247	9.243	
	Fictitious factors	7.67	2	3.835				
C_2HCl_3	Water depth	154.729	2	77.365	15.85	19.247	9.243	*
	HRT	180.994	2	90.497	18.698	19.247	9.243	*
	Current velocity	22.306	2	11.153	2.304	19.247	9.243	
	Fictitious factors	9.68	2	4.84				
C_2Cl_4	Water depth	238.703	2	119.352	12.986	19.247	9.243	*
	HRT	452.319	2	226.16	24.607	19.247	9.243	**
	Current velocity	18.382	2	9.191	1	19.247	9.243	
	Fictitious factors	18.382	2	9.191				

SS, sum of squares; DOF, total degree of freedom; MS, mean square; F_{c1}, critical F value at α level of 0.05; F_{c2}, critical F value at α level of 0.1; Sig, significance (** Extremely significant influence $p<0.05$; * Significant influence $p<0.1$)

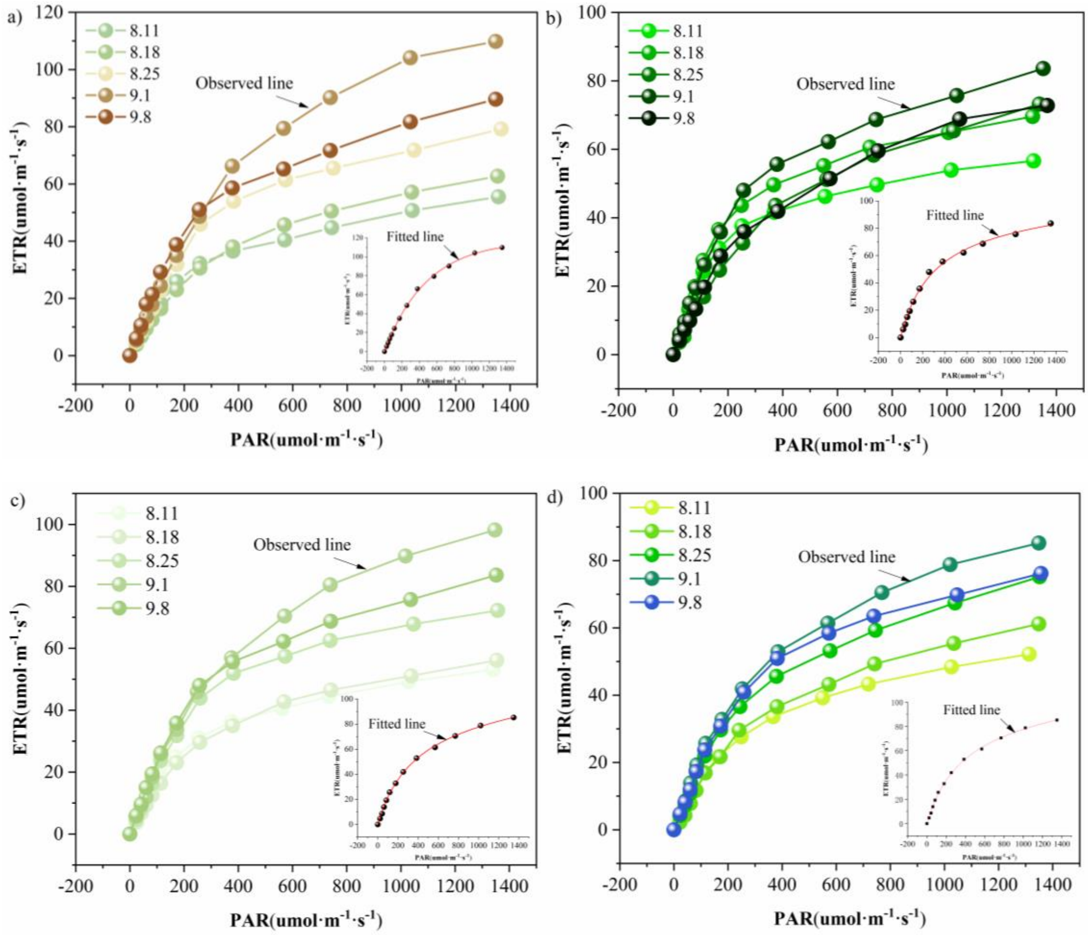
As seen in Table.4, when the water depth was 0.4 m, the HRT was 5d and the current velocity was 1 m/s, VHCs removal efficiencies could reach favorable value. The factor of HRT was the most influential factor in the removal efficiency of $CHCl_3$, CCl_4 and C_2Cl_4, and water depth showed an extremely notable role on the $CHCl_3$ removal in the experiment ($p<0.05$). The sufficient contact time between the reactor and wastewater was directly affected by HRT, which involved in alter the accumulation of aquatic ecosystems in reactor, and the value of it should be extended appropriately to promote the good performance (Su et al., 2009). Besides, the factor of water depth also showed extremely significance on $CHCl_3$ removal ($p<0.05$) and exhibited significant influence on CCl_4, C_2HCl_3, and C_2Cl_4 ($p<0.1$). Water depth was used as a measure of treatment volume, which related to not only the total water volume, but also influence the active quantity of the in-situ restoration system. Mathon et al (2019) reported that the degradation of micropollutants were significant decrease corresponding to the increase of water level, which was in line with our results. And the effect of reactor existed a possible link with the light intensity, which was affected by the water depth. The appropriate light intensity was conducive for promoted potential function on the plants, such as biological function and metabolism status. The plants grow particularly well with the relatively low water depth, the removal of pollutants could be accelerated when the optimal condition was obtained.

Current velocity had a significant influence on the $CHCl_3$ removal, but it was not obvious on the others pollutants removal effects. Some previous studies showed that a low current velocity was promoted to flocculate and precipitate of pollutants in damaged river, which was conducive to the removal of pollutants (Navarro-Ortega et al., 2010; Pan et al., 2016). The variation on the current velocity affected the reactor by altering the degree of architecture of submerged macrophytes, influencing the association on leaf-water interface and adjusting the attachment of bacteria, comprehensively. The significant influence of current velocity was relatively low might be the distribution of VHCs was relatively stable under low current velocity. In addition, both sum of squares and R value of fictitious factors was relatively low indicated that no significant interaction effects existed among the variables (Xiong et al., 2020). The situation mainly because extended HRT, decreased current velocity could strengthen interaction between the restoration area and polluted river water, which was conductive to the adsorption of VHCs through plants and optimizing mass transfer by epiphytic biofilms (Li et al., 2018). The decrease on current velocity would weaken the influence on flow shear, adjusting the attachment and distribution of biofilms. The increase of water depth increased the treatment volume with in situ restoration reactor, exacerbated the formation of anaerobic environment and the hydraulic dead zone (Liu et al., 2016), which would cause the removal efficiency improve more difficult.

In addition, the F value in the variance analysis showed that HRT and water depth had a significant impact on the VHCs removal, and the order of the factors could be summarized as HRT, water depth and current velocity for in situ restoration reactor. The evaluation index of orthogonal test was contact with its impact on the results, which exhibited positively correlation on the two indexes. Both HRT and water depth exhibited the highest significance on the VHCs removal, but current velocity had a bad correlation. However, the accuracy of orthogonal experiment seemed to be lower than single factor analyses, which was the unavoidable disadvantage of this scheme. The reason for the situation could be attributed to the restriction of the level setting (Guo et al., 2017). Considering the shortcomings of the orthogonal test, the further research should be added parallel experiment to improve the accuracy of orthogonal experiment.

3.2. Photosynthetic fluorescence characteristics upon plants

The photosynthesis ability of plants was a drive factor that regulate the growth and metabolism, implied the net assimilation rates of plants, which characterized the plant stress responses at the same (Zhang et al., 2020b). Rapid light curves (RLC) are plots of mechanisms of photosynthesis for 10s, it could reflect the influence of differ environmental factors on the photosynthetic reactions (White & Critchley, 1999). The plots were measured with DIVING-PAM-II, the results were shown in the Fig.5.



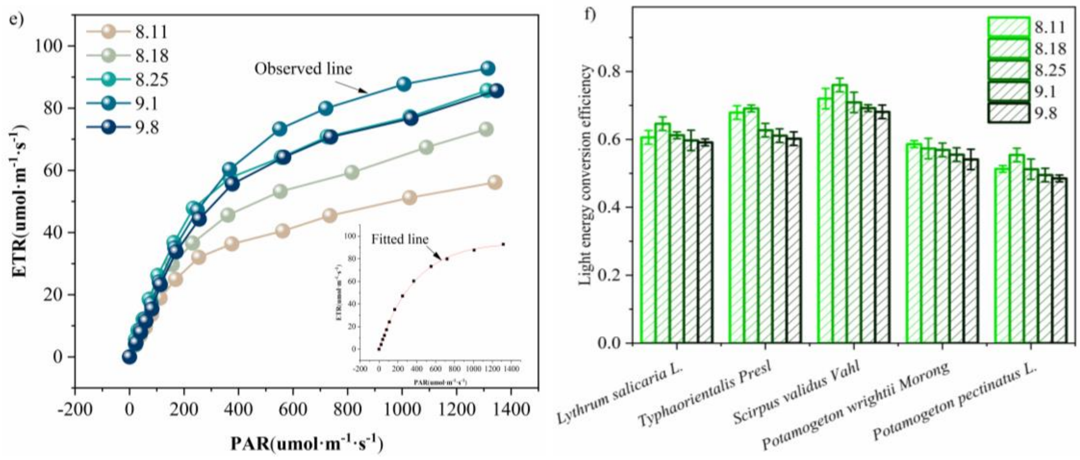


Fig.5 Photosynthetic fluorescence characteristics upon plants (a. *Lythrum salicaria L.* b. *Typhaorientalis Presl* c. *Scirpus validus Vahl* d. *Potamogeton wrightii Morong* e, *Potamogeton pectinatus L.* f. Light energy conversion efficiency)

As is shown in Fig.5, the RLCs of different plants were obtained under similar temperature (20°C), which indicated the plants existed some difference at the intrinsic characteristics of photosynthetic ability and electron transport, especially in stressful environment. On the whole, the relative electron transfer efficiency (ETR) of plants increased with the increase of photosynthetically active radiation and reaction time at the on the peak value, and then decrease slightly. This phenomenon on plants caused by the physiological mechanism for the adaption to environmental stress. The increase in ETR for the several plants were considerably and the values of *Lythrum salicaria L.* was the highest, which exhibited it had the excellent pollutant resistance. The variation on ETR was linked with the adjustment on growth characteristics and photosynthesis pigments for plants (Yaghoubian et al., 2016). The VHCs damaged the photosynthetic apparatus was a cumulative process in plants, which were consistent with the change on RLCs under different experiment plants in our experiment (Hou et al., 2018). In addition, five kinds of experimental plants existed significance difference ($p<0.01$), which supported that prominent diversity exists in the adaptation abilities for different types of plants.

As is shown in Fig.5-f), it was found that the F_v/F_m values of experiment plants were decline with the time extended, which showed the physiological state of the plant was inhibited to a certain extent. The variation on the F_v/F_m value was related to the conversion efficiency of photosystem II, which was linked with non-cyclic electron transport rates and implied the assimilation capability of plants with the help of light energy (Takahashi & Badger, 2011). The decline of F_v/F_m value supported that there existed some damage in the photosynthetic machinery of experiment plants. In addition, Krall et al (2010) proposed the F_v/F_m value near 0.8 was conducive for exploited full potential on the photosynthetic activity and productivity of plants. All the F_v/F_m valve of experiment plants lower than 0.8, which was demonstrated the results that physiological state of the plants was endangered.

Using the presetting program of fluorescence induction curves and light curves, the parameters of the photosynthesis were obtained. According to the according to the model of Frankenbach and SeroDio (2017), the best RLCs of five kinds of experiment plants were fitted by the Eq. (7).

$$ETR = \frac{PAR}{\mathrm{a} \cdot \mathrm{PAR}^2 + \mathrm{b} \cdot \mathrm{PAR} + \mathrm{c}} \tag{7}$$

And the detailed parameters of curves included the maximum photosynthetic electron transfer rate *(ETR_{max})*, the initial slope (α) and half-saturation light intensity (I_k) was calculated by the Eq. (8), Eq. (9) and Eq. (10), respectively.

$$\alpha = \frac{1}{c} \tag{8}$$

$$ETR_{\max} = \frac{1}{b + 2 \cdot \sqrt{a \cdot c}} \tag{9}$$

α—Initial slope, *electrons/photons*

ETR_{max}—Maximum electron transfer rate, $\mu mol \cdot m^{-2} \cdot s^{-1}$

The relevant parameters were carried out through above mathematical calculation, and the results were shown in the Table.5.

Table 5 Detailed parameters of rapid light curves equation upon experiment plants

Name	Fast light curve equation	α	ETR_{max}
Lythrum salicaria L.	$E = \frac{P}{1.039E^{-6} \cdot P^2 + 0.005 \cdot P + 4.013}$	0.249	211.421
Typhaorientalis Presl	$E = \frac{P}{1.615E^{-6} \cdot P^2 + 0.006 \cdot P + 3.836}$	0.261	171.238
Scirpus validus Vahl	$E = \frac{P}{5.562E^{-8} \cdot P^2 + 0.007 \cdot P + 3.82}$	0.262	137.175
Potamogeton wrightii Morong	$E = \frac{P}{6.411E^{-7} \cdot P^2 + 0.01 \cdot P + 3.67}$	0.272	101.423
Potamogeton pectinatus L.	$E = \frac{P}{4.331E^{-7} \cdot P^2 + 0.01 \cdot P + 3.111}$	0.321	95.788

As shown in Table. 5, by comparison, the parameters of RLCs were obvious differences between the different plants. α was the initial slope of the curve, which mean the efficiency of light energy utilization. According to the parameters in the Table.5, the value of *Potamogeton pectinatus L.* was highest, and the value of *Potamogeton wrightii Morong* was the second largest, which exhibited that they had the strongest tolerance to strong light. The phenomenon supported that the two kinds of macrophyte with submerged structure could growth healthily in the water. ETR_{max} is a measure of maximum electron transfer rate, which was related to the calvin cycle metabolism and showed the vitality of plants (San Bautista et al., 2011). The ETR_{max} value of *Lythrum salicaria L.* was highest in our test, which accord with our observation. It might be provided indirect information about the physiological condition of plants, which hinted the plant had superior stress resistance (Brestic & Zivcak, 2013).

3.3. The microbial community structures

The removal of VHCs in in situ restoration reactor mainly relies on biological function, which was similar with the pollutant removal role on constructed wetland (Tang et al., 2020). in situ restoration reactor of different plants and substrates can induce changes in microbial community structure, which influenced on the energy and complex compounds transformation efficiencies. Give full consideration to the characteristics of the fillers, the samples of ceramsite and zeolite were collected during the experiment and analyzed in the end. The results were shown in the Fig.6.

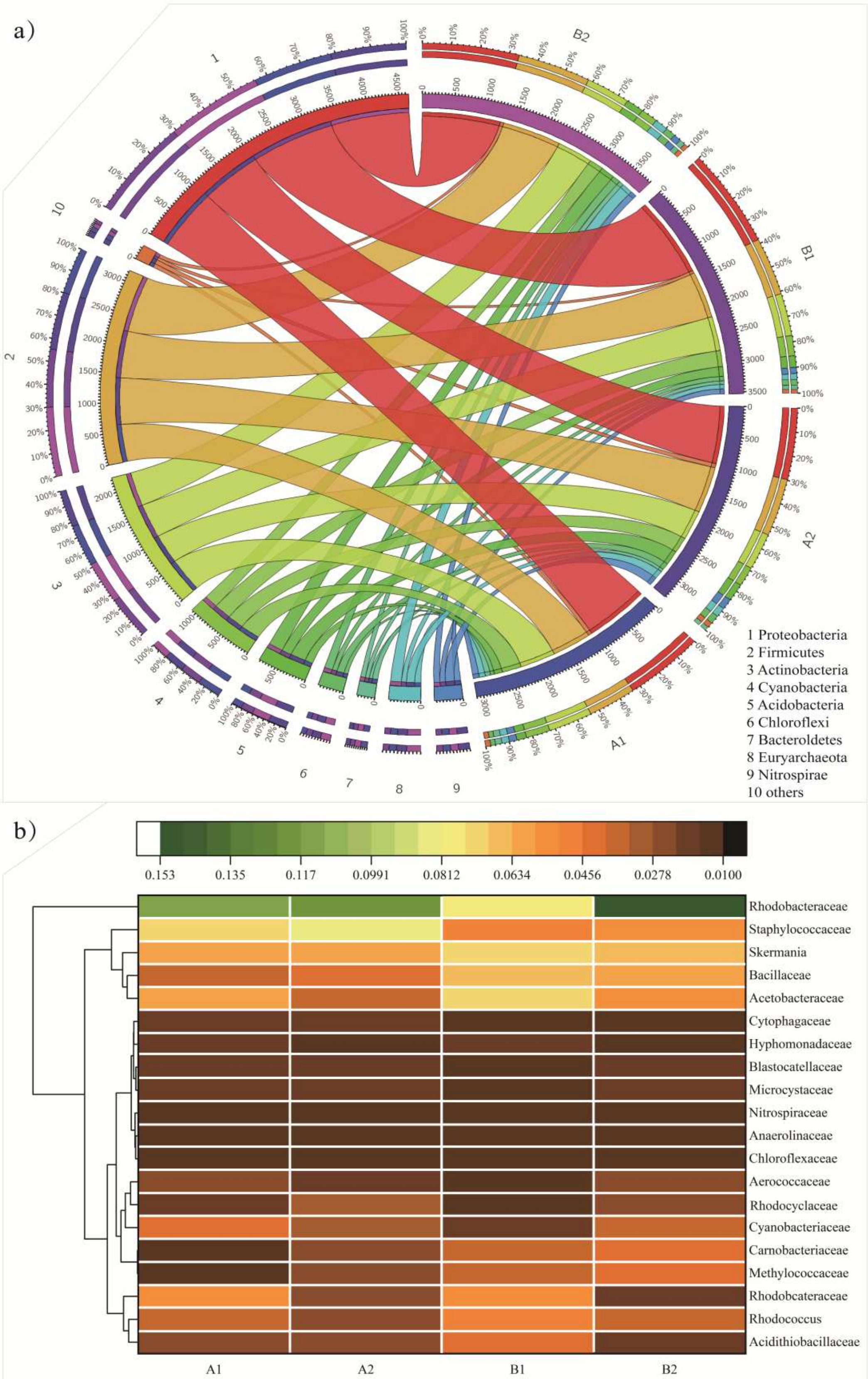


Fig.6. Relative abundance of the main microbial composition (a) phyla level (b) Family level

The distribution of microorganisms at phyla level in fillers are shown in the Fig.6. A1 and A2 are ceramsites at the 9d and 27d, B1 and B2 are zeolites at the 9d and 27d, and a total of 3101, 3320, 3530, 3805 OTUs were identified from the four samples, respectively. Generally, 10 phyla of the bacterial communities were contained in the samples. The biomass samples taken from the in-situ restoration reactor were dominated by *Proteobacteria*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria* respectively at the phylum level, which was account for over half of the microbial community. These microbial communities were always play an important role in the degradation of organic matter, which had been found in the rhizosphere zone usually (Li et al., 2020a; Man et al., 2020). *Proteobacteria* and *Firmicutes* had been reported that included various bacteria with hydrolysis and could degrade complex compounds, which was common flora of macrophyte rhizospheres in the fillers of wetland (Huang et al., 2020; Yang et al., 2020). *Cyanobacteria* was a common component of soil-root interface in wetland substrate, in which some species involved in nutrients removal (Mandal et al., 1992). Besides, *Actinobacteria* were quotidian microorganisms in wetland ecosystem, whose existence was advantageous to the recycling of refractory biomaterials (Hassan et al., 2017).

As is shown in the Fig.6-a), after 18 days of operation of the in-situ restoration reactor, the distribution of biomass was more balanced both in zeolites and ceramsites. There are some previous researches supported that the microbial community structure would be evolved in order to constantly adapt to the variation of the environment, which might be related to the decrease of *Proteobacteria* (Kobayashi et al., 2020; Zheng et al., 2020). Some of *Acinobacteria* had specific degradation capacity, such as *Streptomyces*, *Rhodococcus*, which could immensely helpful for the degradation of recalcitrant compound (Fatahi-Bafghi, 2019). The decrease of the relative abundance of *Actinobacteria* may be related to the acidogenic decomposition process on the VHCs during the experiment. *Firmicutes* contained many microbes, which was responsible for the structure on the bacterial colony. The increase of *Firmicutes* implied that the stability of bacterial biofilm was improved in the fillers (Chouari et al., 2005). Additionally, the content of *Chloroflexi* increased in the fillers, whose existence was advantageous to the dichlorination process in the reactor. In fact, *Firmicutes* and *Chloroflexi* contained a number of microorganisms had with the potential to biodegrade VHCs, such as *Cyanobacteria*, *Bacillus* and *Staphylococcus*, and the change of the two phylum was conducive to the improvement of in situ restoration reactor performance (Guo et al., 2020; Yuan et al., 2017). According to the changes of *Bacteroidetes*, it was founded that there existed different on the content of zeolites and ceramsites. Wolinska et al (2017) found *Bacteroidetes* could be used as a biological index of microbial community structure, and the amount of it would increase when the bacterial community encountered poor environment. And the amount of *Bacteroidetes* increased in the zeolites was conformed to the characteristics of materials, the fillers could adsorb considerable VHCs. And because the zeolites had better adsorption capacity, generous VHCs would accumulate on this filler, which exacerbated the difficult on the living of microorganisms. In addition, some researchers have been reported that *Acidobacteria*, *Cyanobacteria* and *Nitrospirae* were essential factors in wetland substrate, which could maintain the function of wetland (Li et al., 2020b). All these floras were observed as the dominant bacteria in experiment reactor, while the proportion of these phylum were declined in the later period of experiment. It was proved that there existed certain adjustment on the microbial community to suit the change of the influent.

As is shown in the Fig.6-b), 20 species were determined as dominant bacteria, numbers from *Rhodobcateraceae*, *Staphylococcaceae*, *Skermania* were most frequently detected at the family level. Some previous research has been reported that some bacterial strains belong to *Rhodobcateraceae* showed excellent capacity to biodegrade a wide range of organic compound, which were used as bioremediation (Cappelletti et al., 2020). It was the most abundant family in zeolites and ceramsites, no matter in initial or last stage during the experiment. *Staphylococcaceae* was the second most abundant phylum in our results and the relative abundance of *Skermania* was followed closely. *Staphylococcaceae* and *Skermania* included a group of microorganisms had nutrients removal capacity, often as a basic component of activated sludge or wetland structure (Milobedzka & Muszynski, 2015; Wang et al., 2016). Furthermore, *Methylococcaceae* was predominance bacterial group, some researchers demonstrated that it contained some types methanotrophs that could utilize the methane to gain energy (Albers et al., 2015). The proportion of *Methylococcaceae* at the family level was promoted obviously, which was probably related to the affluent CH_4 originated from the reductive action on the VHCs decomposition.

Additionally, both the number of *Bacillaceae* and *Cytophagaceae* exhibited that the opposite trends in the two fillers, which showed the enrichment in the ceramsites and decline in the zeolites. It appeared be link with the active bacteria were affected by the distribution on the pollutant concentration. The relative abundance of *Chloroflexaceae* was clearly more abundant in the later stage on the experiment. It has been widely reported that *Chloroflexaceae* was a kind of photosynthetic bacteria, which might be live close vicinity to the primary producers and along with the abundance around the roots (Nubels et al., 2002). We believe the positive change on it connected with the diffusion on the plant roots. In addition, the number of *Rhodocyclaceae* in the fillers increased, which degraded trichloroethene in the in-situ restoration reactor and could assist dechlorinate carbon tetrachloride to form CH_4 and CO_2 (ZivEl et al., 2011). The abundance of family *Skermania* and *Rhodococcus* decreased slightly, both of the two kinds of microorganisms from the phylum *Acidobacteria*, which is consistent with some previous studies that *Acidobacteria* was difficult to use VHCs cometabolism (Maaike et al., 2015).

Overall, the results showed that the change of polluted river water led to the change of microbial community structure. In fact, the microbial communities would make a great response to adapt to the changes of environment, which was in line with the previous experimental results, and this situation could be described to two points. For one view, the microenvironment was regulated by rhizosphere of aquatic macrophytes. For another, the microorganisms were stimulated by the actual polluted river water, which would enhance community diversity, especially under a relatively high nutrient status (Navel et al., 2012; Zhang et al., 2010). But on the whole, the dominant microbial community stay relatively stable under the diverse hydraulic conditions, which help the degradation and conversion of the VHCs and was essential to the dechlorinating function of the in-situ reactor.

3.4. Mechanism of VHCs degradation

VHCs degradation needed multiple interactions, particularly in the in-situ restoration reactor. In-situ ecological restoration reactor was adopted to treat the VHCs in polluted river water, the processes were shown in the Fig.7.

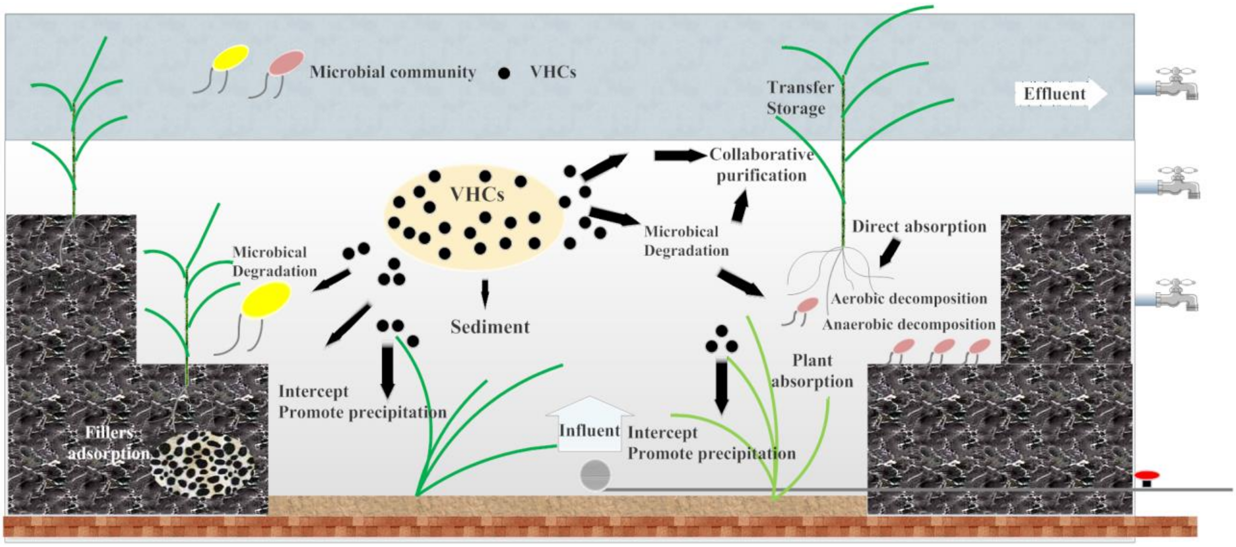


Fig.7 Degradation mechanism of VHCs in reactor

The material and energy cycle of in-situ remediation reactor ecosystem was the core of its function exertion. The degradation reaction process of dehalogenation in plants were complex. Under the action of peroxidase, the conversion of VHCs was usually completed by substitution reaction. In this experiment, the purification effect of plants depended on the process of gas deposition and particulate matter deposition. On the one hand, through the exchange of gas deposition between the pores and the liquid surface, the migration trajectory of pollutant particles was changed. On the other hand, the effect of hydraulic shock could be reduced by macrophyte and the settlement of pollutant particles could be promoted by the way of particle sedimentation.

The micropores structure of the filler was helpful to adsorb the small molecule pollutant particles, which cause the separation of VHCs from volatile organic compounds (Li et al., 2020c). In addition, adsorption is a self-energy reduction process. According to the second law of thermodynamics, system has a tendency to reduce its energy, which accelerated the pollutant molecular was absorbed in the rough surface of zeolite and ceramsites (Long et al., 2018).

Actually, the bacteria colonies are generally favoured the anaerobic environment to finish dichlorination process (Jagnow et al., 1977). In terms of microbial remediation, first of all, the substitution reaction was the main degradation avenue of VHCs by microorganisms under anaerobic conditions. The redox potential of VHCs containing halogen was relatively high (260-570mV), and it was more likely to become the electron acceptor of dehalogenated microorganisms in anaerobic environment. VHCs compounds got electrons and halogen atoms were replaced by hydrogen atoms to complete reduction and dehalogenation reaction. Or the dechlorination is an inducible process depended on the functional bacteria for broken the C-Cl bonds, formed the C-H bonds and finished biodegradation process (Dong et al., 2018). The basic processes might be shown in the Eq. (10), Eq. (11), Eq. (12), Eq. (13), Eq. (14), and Eq. (15):

$$CCl_4+2H_2O \rightarrow CHCl_3+Cl^-+H^++HCOOH \tag{10}$$

$$2CHCl_3+2H_2O \rightarrow CH_2Cl_2+HCOOH+2Cl^-+2H^+ \tag{11}$$

$$2CH_2Cl_2+2H_2O \rightarrow CH_3Cl+HCOOH+3Cl^-+3H^+ \tag{12}$$

$$2CH_3Cl+2H_2O \rightarrow CH_4+HCOOH+2Cl^-+4H^+ \tag{13}$$

$$2C_2HCl_3+4H_2O \rightarrow 2CO_2+C_2H_4+6Cl^-+6H^+ \tag{14}$$

$$2C_2Cl_4+4H_2O \rightarrow 4CO_2+8Cl^-+8H^+ \tag{15}$$

4. Conclusions

Through outdoor simulation testing, in in situ restoration method was applied to degrade VHCs of polluted river water, which showed that it was feasible. Favorable removal effect of $CHCl_3$, CCl_4, C_2HCl_3 and C_2Cl_4.could be obtained through in situ ecological restoration. In the experiment, orthogonal tests were applied to evaluated different influencing factors. The findings in this research suggested that when the water depth was 0.4 m, the HRT was 5d and the current velocity was 1 m/s, VHCs removal efficiencies could achieve favorable value, the removal efficiency of $CHCl_3$ CCl_4, C_2HCl_3 and C_2Cl_4.could reach 70.27%, 70.59%67.74% and 81.82%, respectively. Besides, F test results exhibited that HRT and water depth were significantly related to the removal efficiency of reactor. The RLCs of plants were analyzed by DIVING-PAM-II, which prominent diversity exists in the adaptation abilities for different types of plants, but the physiological state of the plants was endangered by VHCs of polluted river water in all.

Moreover, the microbial community structure and diversity of reactor were regulated to more balance and adapt to the variation characteristics of complex polluted river water conditions. The microbial community structures of fillers were tested by high-throughput sequencing, the findings supported that the proportion on the reductive dechlorination bacterial of VHCs was improved. And there existed a slightly improvement on the relative abundance of *Rhodocyclaceae*, which might be related to it could promote VHCs degradation. In general, in situ ecological restoration had a good potential performance on pollutants removal in polluted river water. The results showed that technology provided a green and low consumption approach for the maintenance and purification on polluted river water.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions:

Jia Wang: Validation, Visualization, Software, Data curation, Writing-Original Draft.

Shuangrong Wu: Supervision, Visualization, Writing-Review & Editing..

Qi Yang: Conceptualization, Methodology, Software, Investigation, Writing-Review & Editing.

Yonggang Gu: Resources, Formal analysis, Supervision.

Peijing Wang: Investigation, Data curation.

Zhaoxin Li: Writing-Review & Editing.

Lei Li: Software, Investigation.

Thank you.

Yours sincerely,

Yonggang Gu

Ethical approval

Not applicable.

Consent to Participate

I declare here that all authors approve publish this paper.

Thank you.

Yours sincerely,

Yonggang Gu

Competing Tests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials

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