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 toxicity and persistence 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 experiments showed that when the water depth was 0.4 m, the HRT was 5 days, and the current velocity was 1 m/s, the optimal removal efficiency of VHCs in the reactor was achieved. And the removal rates of CHCl3, CCl4, C2HCl3, and C2Cl4 reached 70.27%, 70.59%, 67.74%, and 81.82%, respectively. The results showed that both HRT and water depth were significantly related to the removal efficiency of reactor. The physiological state of the plants was analyzed by fitting rapid light curve (RLC) model, which showed that the accumulation of VHCs inhibited the photosynthetic performance of plants. Moreover, the microbial community structures of fillers were tested by high-throughput sequencing, and the findings supported that the microbial community made a great response to adapt to the changes in 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 a potential approach for removal VHCs in polluted river water.

Keywords Volatile halogenated hydrocarbons · Orthogonal tests · In situ ecological restoration · Microbial community

Introduction

Urbanization in China has accelerated the transformation of geomorphological features and ecological environment in urban rivers, which includes the decrease in hydrological connectivity and the pollutants accumulated at the bottom of river channels (Wang et al. 2017). Human activity changes the original shape of the river and injects additional pollutants thus affecting the water quality frequently (Hirsch et al. 2010). With the process of urbanization continues to move forward, some attentions were applied to urban water environment, especially in urban rivers and lakes (Lu et al. 2018). Volatile halogenated hydrocarbons (VHCs) are 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 the air environment as a consequence of low boiling points, adding air pollutants and the local people risk of illness in urban areas (Mun and Townley 2021). VHCs have been widely accepted as a major source of photochemical smog since the beginning of the twenty-first century. Besides, variety of VHCs produced by plastic petroleum and landfill leachate influx into urban river altering the characteristics of water environment and initiation potential
adverse effects on humans (Wang et al. 2020). VHCs would cause continuous hazard in environmental media, which was particularly sensitive and prominent in river within the city limits.

River pollution caused by human activities is very common in the world, and many regions and countries begin to show concerns for river quality and take measures to treat it. As we all know, numerous factors, such as water depth and current velocity, regulate actual situation of the river and affect migration status and pollutant distribution in damaged rivers (Jiang et al. 2017; Rugner et al. 2019). Due to the complex characteristics of river pollution, 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 representative eco-engineering plants are an important component of the river ecosystem, which have capable of influencing various biotic and abiotic environments, including river status (Zhang et al. 2018; Özgencil et al. 2020). Slope wetland system has characterized by excellent geomorphology adaptability and flexible controllability, which was suitable for recovery and remediation of damaged rivers (Wang et al. 2021). Aimed at maintenance and restoration of polluted river water, the two technologies need to be a 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 of the organic matter in the polluted river water. Additionally, both Submerged macrophytes and slope wetland are biological systems involved in the degradation of organic matter. Plants in conjunction with microflora in the system would play cleaning function on the coupling system by the bioremediation function (Kushwaha et al. 2018). Hence, the regulation on plant physiological activities and microbial community status is of a vital concern, which involves 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 promotes water resources management (Song et al. 2017). Many river restoration projects have been applied to urban river remediation through the last few years, but the degradation of VHCs in polluted river water has been rarely reported. In view of VHCs being toxic and persistent in the environment, determining its content of polluted river water and proposing appropriate measures to restore the river ecosystem are 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 of VHCs in the polluted river water was investigated. We hypothesized that the different VHC purification effects would significantly different in response to the different types of hydraulic conditions. Hence, multifactor orthogonal experimental design was designed to select and explain the factors mostly influencing the wastewater treatment performance of in situ restoration reactor, and determine the optimal operating conditions to optimize the removal of VHCs in reactor in this study. The variation on the physiological state of plants was checked by the underwater modulation chlorophyll fluorescence. Besides, the change of substrate microorganism was measured before and after the experiment to evaluate the flora on the dichlorination reaction of VHCs. The pollutant decomposition mechanisms in the reactor were also comprehensively analyzed.

**Materials and methods**

**Experimental device**

To assure an effective and authentic research, the experiment was carried out outdoors. As shown in Fig. 1, one device was set up in the experiment, which dimension was 1-m high, 2-m wide, and 7-m 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 0.4 m, 0.6 m, and 0.8 m (to the bottom). Slope wetland contained three types of plants (ScirpusvalidusVahl, Typha orientalis Presl, and Lythrum salicaria L.), which were planted from bottom to up, respectively. Potamogeton wrightii Morong and Potamogeton pectinatus were closely adjacent seeded around the ScirpusvalidusVahl in the sediment of the reactor. And the planting density of five kinds of plants was set at 30clumps/m2. The pushing flow device (QJB0.85) was purchased from Sapphire environmental protection (Nanjing, China), and the impeller diameter of which was 220 mm. 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 was directly extracted from the polluted river by water pump, which enhance the reliability of the simulation experiment and guarantee the authenticity of the results to a certain extent.

**Experimental chemical and instrument**

The guaranteed reagent (GR) methanol was selected as diluent of the standard solutions, which was purchased from Beijing chemical plant (Beihua, China). Standard solutions (GSB07-1982–2005) of 1000 μg mL⁻¹ VHCs in ethanol were purchased from standard sample Institute of
Ministry of environmental protection, including CHCl₃, CCl₄, C₂HCl₃, and C₂Cl₄. Analytical grade of NaCl was 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). Chloride standard solutions 1000 μg mL⁻¹ (GSB 07–1267-2000) were 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.

**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 were diluted with methanol to obtain the 10 μg mL⁻¹ preparation solutions. Preparation solutions were allowed the headspace extraction and gas-phase operations relatively handy. The NaCl was dried to a constant weight (350 °C for 3 h) 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-, and 50-μL preparation solutions were added up respectively into 10 mL ultra-pure water in the headspace bottle, and the mixture sufficient agitated. 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 were consisted the 111-space autosampler, which could heat samples and convey the bottles into the GC equipment. Chromatographic conditions: initial temperature was 40 °C for 2 min and increased to 100 °C at 60 °C min⁻¹, then 10 °C min⁻¹ to 200 °C. Detection temperature was set as 320 °C. Volatile compounds were separated on an Agilent DB-5 MS column (30 M×0.25 mm, 0.25-μm film thickness), with helium as the carrier gas at a constant flow of 1.0 mL/min. The results are shown in Fig. 2a.

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

**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. The main stream is set with several sewage outlets, and the self-purification capacity of water 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.

The Xiaozhong River receives composite sewage throughout the year, and the pollutants mainly come from the surrounding domestic garbage, sewage treatment plants, and rainfall (Zhang et al. 2020a). The integral state of river was sluggish, and some areas existed stagnant zone. Using the 1000-mL sample bottle directly collected water samples from the experimental site and then analyzed in the laboratory (sampling two times). The characteristics of polluted river water are shown in Table 1.

### Table 1 Characteristics of polluted river water

| Parameter | CHCl₃ (μg L⁻¹) | CCl₄ (μg L⁻¹) | C₂HCl₃ (μg L⁻¹) | C₂Cl₄ (μg L⁻¹) | pH  |
|-----------|----------------|---------------|-----------------|----------------|-----|
| Content   | 0.21           | 0.17          | 0.37            | 0.11           | 5.9~7.1 |

Experimental methods

In this study, in situ restoration effects of polluted river water were evaluated by orthogonal tests under the water depth, HRT, and current velocity as variables. The three factors were selected as influencing factors, containing HRT (1, 3, and 5 days), current velocity (1, 2, and 3 m/s), and water depth (0.4, 0.6, and 0.8 m), and three levels were taken for each factor. The fictitious factors were added to the orthogonal matrix, for avoiding the influence of error caused by stochastic factors. Meanwhile, the methods of outdoor simulation experiments were adopted to the experiment to assure the factuality of results. Water samples were collected in glass sampling bottle (50 mL) and stored at 4 ℃ until analysis. The samples before and after treatment were conveyed by 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.

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 $-20^\circ$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 was completed by illuminamiseq pe300 sequencing platform. Besides, plant physiological performances were tested by underwater modulated chlorophyll fluorometer and the data measured with the instrument were fitted with the RLCs model to determine the transformation of the physiological responses.

Data analysis

Considering the characteristics of wetlands, different HRT was applied to the reactor to meet the requirements of variables. The HRT in the reactor was calculated by Eq. 1.

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

(1)

$V$ is the treatment volume in reactor (m$^3$) and $Q$ is the influent flow in reactor (m$^3$/day).

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

$$SS_i = \frac{1}{r} \sum_{i=1}^{k} K_{ij}^2 - T$$

(2)

$$T = \frac{S^2}{n}$$

(3)

$SS_i$ is the sum of square deviance with each factors; $r$ and $k$ are the repeat times of each level, the number of levels with each factor; $K_{ij}$ is the total indicators of each factor level; $T$ is the sum of all index; and $n$ is the number of experiments ($n = 9$).

$$N = df_a = df_b = df_c = df_d = m - 1$$

(4)

$$MS_i = \frac{SS_i}{df_i}$$

(5)

$N$ is the degree of freedom and $MS_i$ is the 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 identifies the significance on the factors.

$$F_i = \frac{MS_i}{MS_e}$$

(6)

Rapid light curves (RLCs) were described by fitting the model of Eq. (7):

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

(7)

And the detailed parameters of curves included the maximum photosynthetic electron transfer rate ($ETR_{max}$), and the initial slope ($\alpha$) was calculated by Eq. (8) and Eq. (9), respectively.

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

(8)

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

(9)

$\alpha$ is the initial slope, $electrons/photons$, and $ETR_{max}$ is the maximum electron transfer rate, $\mu mol \: m^{-2} \: s^{-1}$.

Results and discussion

The degradation of VHCs under orthogonal experiment

Construction of orthogonal experiment

Transport of pollutants in damaged river is closely associated with hydrodynamics situation on the polluted river water, which included current velocity, river flow, and water depth (Anwar Sadat et al. 2020). In order to maximize the critical factors for influencing the treatment efficiency of reactor and reduce material waste, $L_9(3^4)$ orthogonal experiment was applied in this study. Details of the factors and levels in orthogonal experiment are shown in Table 2.

Orthogonal experiment results

The pollutant removal effect of the reactor showed large fluctuations under different operating conditions. The results

| Table 2 | Factors and levels in the orthogonal experiment |
|---------|-----------------------------------------------|
|         | Water depth (m) | Current velocity (m/s) | Fictitious factors |
| Factors | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| HRT (d) | 1 | 3 | 5 | 1 | 2 | 3 | 1 | 2 | 3 |
| Fictitious factors | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
of the VHC removal efficiency orthogonal experiment are shown in Fig. 4.

As shown in Fig. 4, the removal efficiency of four VHCs was different, which were associated with the change of influencing factors. The average removal efficiency of CHCl₃, CCl₄, C₂HCl₃, and C₂Cl₄ 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 determining the effects on different experimental factors, significance tests of orthogonal experiment results were analyzed. The results are shown in Table 3.

There were significant differences in the tested factors, and the tendency analysis was carried out by formulas in the “Data analysis” section to determine the optimal level of each factor and thus the optimal operating conditions for the removal. 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 are listed in Table 4.

As seen in Table 4, when the water depth was 0.4 m, the HRT was 5 days, and the current velocity was 1 m/s, VHC removal efficiencies could reach a favorable value. The factor of HRT was the most influential factor in the removal efficiency of CHCl₃, CCl₄, and C₂Cl₄, and water depth showed an extremely notable role in the CHCl₃ removal in the experiment ($p < 0.05$). The sufficient contact time between the reactor and wastewater was directly affected by HRT, which involved in altering the activity time for treating pollutants and the pollutant accumulation of aquatic ecosystems in reactor, particularly for relating to the transportation of strongly hydrophobic organic pollutants (Su et al. 2009; Marc et al. 2013). HRT should be extended appropriately to promote the performance improvement of the reactor. Besides, the factor of water depth also showed extremely significance on CHCl₃ removal ($p < 0.05$) and exhibited significant influence on CCl₄, C₂HCl₃, and C₂Cl₄ ($p < 0.1$). Water depth was used as a measure of treatment volume, which was 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 was significantly decreased corresponding to the increase of water level, which was in line with our results. 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 promoting potential function on the plants, such as biological function and metabolism status. The plants grow particularly well with the relatively low water depth, and the removal of pollutants could be accelerated when the optimal condition was obtained.

Current velocity had a significant influence on the CHCl₃ removal, but it was not obvious on the other pollutant removal. 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. Some previous studies showed that a low current velocity was promoted to flocculate and precipitate of pollutants in polluted river water, which was conducive to the removal of pollutants (Navarro-Ortega et al. 2010; Pan et al. 2016). The significant influence of current velocity was relatively low might be because the distribution of VHCs was relatively stable under low current velocity. In addition, both sum of squares and $R$ value of fictitious factors were relatively low indicating that no significant interaction effects existed among the variables (Xiong et al. 2020). The situation mainly because extended HRT and decreased current velocity could strengthen interaction between the restoration area and polluted river water, which was conducive to the adsorption of VHCs through plants and optimizing mass transfer by epiphytic biofilms (Li et al. 2018). The decrease in 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 and exacerbated the formation of anaerobic environment and the hydraulic dead

Fig. 4 The VHC removal efficiency under different influencing factors
zone (Liu et al. 2016), which would cause the removal efficiency improve more difficult.

The numeric size of evaluation index of orthogonal test was main impact on the results, where the larger of the value, the bigger the influence is. As seen in Table 4, both HRT and water depth exhibited the highest significance on the VHC removal, but current velocity had a relatively weak correlation. Besides, the $F$ value in variance analysis showed that the order of the factors could be summarized as HRT, water depth, and current velocity for in situ restoration reactor. 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, further research should be added parallel experiment to improve the accuracy of orthogonal experiment.

| 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                  |

Table 3 Orthogonal matrix and significance test of results

| Water depth (m) | K₁ | K₂ | K₃ | R   |
|-----------------|----|----|----|-----|
| CHCl₃           | 181.08 | 188.23 | 174.19 | 218.19 |
| CCl₄            | 162.16 | 176.47 | 155.59 | 181.83 |
| C₂HCl₃          | 156.76 | 158.82 | 143.99 | 190.92 |
| C₂Cl₄           | 60.36 | 62.74 | 58.06 | 72.73 |

| HRT (d)         | K₁ | K₂ | K₃ | R   |
|-----------------|----|----|----|-----|
| CHCl₃           | 145.95 | 158.82 | 143.99 | 172.74 |
| CCl₄            | 162.16 | 170.58 | 153.67 | 200.01 |
| C₂HCl₃          | 191.89 | 194.12 | 176.11 | 218.9 |
| C₂Cl₄           | 48.65 | 52.94 | 48    | 57.58 |

| Current velocity (m/s) | K₁ | K₂ | K₃ | R   |
|------------------------|----|----|----|-----|
| CHCl₃                  | 172.97 | 176.47 | 155.59 | 190.92 |
| CCl₄                   | 167.57 | 170.58 | 153.67 | 200.01 |
| C₂HCl₃                 | 190.27 | 176.47 | 162.18 | 200.01 |
| C₂Cl₄                  | 5.4  | 5.89 | 10.84 | 9.09 |

| Fictitious factors     | K₁ | K₂ | K₃ | R   |
|------------------------|----|----|----|-----|
| CHCl₃                  | 162.16 | 176.46 | 154.83 | 200.01 |
| CCl₄                   | 167.57 | 170.59 | 156.76 | 190.92 |
| C₂HCl₃                 | 170.27 | 176.47 | 162.18 | 200.01 |
| C₂Cl₄                  | 5.4  | 5.88 | 7.35  | 9.09 |
Photosynthetic fluorescence characteristics upon plants

The photosynthesis ability of plants was a driving factor that regulates the growth and metabolism, implying the net assimilation rates of plants, and the change of the photosynthetic process appraised the plant stress responses and resistance at the same (Zhang et al. 2020b). RLCs are generated by applying short periods (10–30 s) of exposure to each irradiance level, and it could enable the integrated characterization of the photophysiology and reflect the influence of different environmental factors on the photosynthetic reactions (White and Critchley 1999). The plots were measured with DIVING-PAM-II. The results are shown in Fig. 5.

The RLCs of different plants were obtained under similar temperature (20 °C), which indicated the plants existed some differences in the intrinsic characteristics of photosynthetic ability and electron transport, especially in stressful environment. The variation on ETR was linked with the adjustment on growth characteristics and photosynthesis pigments for plants (Yaghoubian et al. 2016). On the whole, the relative electron transfer efficiency (ETR) of plants increased with the increase of photosynthetically active radiation and reaction time at the peak value and then decrease slightly. This phenomenon on plants was caused by the physiological mechanism for the adaption to environmental stress. On the one hand, the increase in ETR for the several plants was considerable, which reflected the success of transplant and growth restoration of the plants. On the other hand, the VHCs damaged the photosynthetic apparatus which was a cumulative process in plants, and it could hurt the plants by changing the enzyme activity (Hou et al. 2018) The ETR values of Lythrum salicaria L. were the highest under the bioremediation experimental conditions, which exhibited it had excellent pollutant resistance. In addition, though the RLCs on five kinds of experimental plants showed similar trends (increased firstly and then decreased), there existed significant differences in the data (p < 0.01), which supported that prominent diversity exists in the adaptation abilities for different types of plants.

As shown in Fig. 5f, it was found that the $F_{v}/F_{m}$ values of experiment plants 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 and 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 (2010) proposed the $F_{v}/F_{m}$ value near 0.8 was conducive for exploiting full potential on the photosynthetic activity and productivity of plants. All the $F_{v}/F_{m}$ values of experiment plants were lower than 0.8, which was demonstrated 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 model of Frankenbach and SeroDio (2017), the best RLCs of five kinds of experiment

| Source of variation | SS     | DOF | MS     | $F$    | $F_{c1}$ | $F_{c2}$ | Sig          |
|---------------------|--------|-----|--------|--------|----------|----------|--------------|
| **CHCl$_3$**        |        |     |        |        |          |          |              |
| Water depth         | 108.732| 2   | 54.374 | 45.466 | 19.247   | 9.243    | **          |
| HRT                 | 361.902| 2   | 180.95 | 31.83  | 19.247   | 9.243    | **          |
| Current velocity    | 914.899| 2   | 45.744 | 9.559  | 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$_2$HCl$_3$**    |        |     |        |        |          |          |              |
| 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   | 11.536 | 2.304  | 19.247   | 9.243    |              |
| Fictitious factors  | 9.68   | 2   | 4.84   |        |          |          |              |
| **C$_2$Cl$_4$**     |        |     |        |        |          |          |              |
| 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  |        |          |          |              |

**Table 4** ANOVA of the orthogonal experimental results

SS, sum of squares; DOF, total degree of freedom; MS, mean square; $F_{c1}$, critical $F$ value at $\alpha$ level of 0.05; $F_{c2}$, critical $F$ value at $\alpha$ level of 0.1; Sig, significance (**Extremely significant influence $p<0.05$; *Significant influence $p<0.1$)
plants were fitted by Eq. (7). The relevant parameters were carried out through above mathematical calculation, and the results are shown in Table 5. As shown in Table 5, by comparison, the parameters of RLCs were obvious differences between the different plants. \( \alpha \) was the initial slope of the curve, which mean

| Name                           | Fast light curve equation | \( \alpha \) | \( ETR_{\text{max}} \) |
|-------------------------------|--------------------------|-------------|----------------------|
| Lythrum salicaria L           | \( E = 1.039E^{-6} \cdot P + 0.005 \cdot P + 0.013 \) | 0.249       | 211.421              |
| Typhaorientalis Presl         | \( E = 1.615E^{-6} \cdot P + 0.006 \cdot P + 3.836 \) | 0.261       | 171.238              |
| Scirpus validus Vahl          | \( E = 5.562E^{-7} \cdot P + 0.007 \cdot P + 3.826 \) | 0.262       | 137.175              |
| Potamogeton wrightii Morong   | \( E = 6.411E^{-7} \cdot P + 0.01 \cdot P + 3.673 \) | 0.272       | 101.423              |
| Potamogeton pectinatus L      | \( E = 4.311E^{-7} \cdot P + 0.01 \cdot P + 3.111 \) | 0.321       | 95.788               |
the efficiency of light energy utilization. According to the parameters in Table 5, the value of Potamogeton pectinatus L. was the 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 macrophytes with submerged structures could grow healthily in polluted river water. \( ETR_{\text{max}} \) 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 \( ETR_{\text{max}} \) value of Lythrum salicaria L. was highest in our test, which accords with our observation. It might be provided indirect information about the physiological condition of plants, which hinted the plant had superior stress resistance (Brestic and Zitveck 2013).

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 the constructed wetland (Tang et al. 2020). The in situ restoration reactor of different plants and substrates can induce changes in microbial community structure, which influenced the energy and complex compound transformation efficiencies. The samples of ceramsite and zeolite were gathered during the experiment and analyzed in the end. The results are shown in Fig. 6.

The distribution of microorganisms at phyla level in fillers is shown in 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, and 3805 OTUs are 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 playing 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 were a common component of soil-root interface in wetland substrate, in which some species involved in nutrient 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 shown in Fig. 6a, after 18 days of operation of the in situ restoration reactor, the distribution of biomass is more balanced both in zeolites and ceramsites. There are some previous researches supporting that the microbial community structure would be evolved 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 capacities, such as Streptomyces and Rhodococcus, which could be immensely helpful for the degradation of recalcitrant compounds (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 were responsible for the structure of 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. Firmicutes and Chloroflexi contained many microorganisms which had the potential to biodegrade VHCs, such as Cyanobacteria, Bacillus, and Staphylococcus, and the change of the two phyla 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 found that there existed differences in the content of zeolites and ceramsites. Wolinska et al (2017) found that 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. The amount of Bacteroidetes increased in the zeolites was conformed to the environmental deterioration triggered by the accumulation in fillers of the VHCs, which exacerbated the difficulty on the living of microorganisms. Ma et al (2011) demonstrated the existence of VHCs could inhibit some types of microorganisms (e.g., Proteobacteria, Actinobacteria) to assist in sifting hydrogen production bacteria, which was caused the negative influence on the certain microbe. 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 phyla declined in the later period of experiment. It was proved that existed certain adjustments on the microbial community to suit the change of the influent.

As shown in Fig. 6b, 20 species were determined as dominant bacteria, and numbers from Rhodobacteraceae, Staphylococcaceae, and Skermania were most frequently detected at the family level. Some previous research has been reported that some bacterial strains belonging to Rhodobacteraceae showed excellent capacity to biodegrade a...
Fig. 6 Relative abundance of the main microbial composition: (a) phyla level and (b) family level
wide range of organic compounds, 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 family in our study and the relative abundance of *Skermania* was followed closely. *Staphylococcaceae* and *Skermania* included a group of microorganisms that had nutrients removal capacity, often as a basic component of activated sludge or wetland structure (Milobedzka and Muszynski 2015; Wang et al. 2016). Furthermore, *Methylococcaceae* was predominance bacterial group, and some researchers demonstrated that it contained some types of 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 CH₄ originating from the reductive action on the VHC decomposition.

Additionally, both the *Bacillaceae* and *Cytophagaceae* exhibited opposite trends in the two fillers, which showed the enrichment in the ceramsites and decline in the zeolites. It appeared to be linked with the active bacteria being affected by the distribution of the pollutant concentration. The relative abundance of *Chloroflexaceae* was clearly more abundant in the later stage of 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 (Nubel 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 help trichloroethene degradation in the in situ restoration reactor and could assist dechlorinate carbon tetrachloride to form CH₄ and CO₂ (Ziv-El 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* had difficulty to use VHC cometabolism (Maulik et al. 2015).

Overall, the results showed that the change of polluted river water led to the change of microbial community structure, and some enrichment in dechlorination microorganisms occurred to some extent. Actually, the microbial communities would make a great response to adapt to the changes of environments, which was in line with the previous experimental results, and this situation could be described to two points. For one view, the microenvironment was mainly 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 communities stay relatively stable under the diverse hydraulic conditions, which help the degradation and conversion of the VHCs and were essential to the dechlorinating function of the in situ reactor.

**Mechanism of VHC degradation**

VHC degradation is a result of multiple interactions, particularly in the in situ restoration reactor. In situ ecological restoration reactor was adopted to treat the VHCs in polluted river water, and the processes are shown in Fig. 7.

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 was 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 micropore 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. The protrusion of adsorbent is surrounded by air phase while the sunken position is fixed by solid phase, and the interatomic force in solid phase is stronger than that in the gas phase. According to the second law of thermodynamics, system trends to reduce its energy, which accelerated the pollutant molecular, were absorbed in the rough surface of zeolite and ceramicsites (Long et al. 2018).

Actually, the bacterial colonies are generally favored in 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 (260–570 mV), and it was more likely to become the electron acceptor of dehalogenation microorganisms in anaerobic environment. VHC compounds got electrons and halogen atoms were replaced by hydrogen atoms to complete reduction and dehalogenation reaction. Or the dechlorination is an inducible process depending on air phase while the sunken position is fixed by solid phase, and the interatomic force in solid phase is stronger than that in the gas phase. According to the second law of thermodynamics, system trends to reduce its energy, which accelerated the pollutant molecular, were absorbed in the rough surface of zeolite and ceramicsites (Long et al. 2018).

The basic processes might be shown in Eq. (10), Eq. (11), Eq. (12), Eq. (13), Eq. (14), and Eq. (15):

\[ \text{2CCl}_4 + 2H_2O \rightarrow \text{CHCl}_3 + 2Cl^- + H^+ + HCOOH \]  
(10)

\[ \text{2CHCl}_3 + 2H_2O \rightarrow \text{CH}_2Cl_2 + HCOOH + Cl^- + 2H^+ \]  
(11)

\[ \text{2CH}_2Cl_2 + 2H_2O \rightarrow \text{CH}_3Cl + HCOOH + 3Cl^- + 3H^+ \]  
(12)

\[ 3\text{CH}_3Cl + 2H_2O \rightarrow 2\text{CH}_4 + HCOOH + 3Cl^- + 3H^+ \]  
(13)

\[ 2\text{C}_2\text{HCl}_3 + 4\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + \text{C}_2\text{H}_4 + 6\text{Cl}^- + 6\text{H}^+ \]  
(14)

\[ \text{C}_2\text{Cl}_4 + 2\text{H}_2\text{O} \rightarrow \text{CH}_2\text{Cl}_2 + \text{CO}_2 + 2\text{Cl}^- + 2\text{H}^+ \]  
(15)

Conclusions

Through outdoor simulation testing, an in situ restoration method was applied to degrade VHCs of polluted river water, which showed that it was feasible. In the experiment, orthogonal tests were applied to evaluate different influencing factors. The findings in this research suggested that the VHC removal efficiency by in situ restoration reactor can be significantly improved via moderate transformation of the hydraulic conditions, due to the improvement of microbial activity and plant growth. Furthermore, the microbial community structures of fillers were tested by high-throughput sequencing and the RLCs of plants were analyzed by DIVING-PAM-II, which revealed a critical role played by plants and microorganism in VHC removal and the real potential of the system for VHC remediation in the field. In general, in situ ecological restoration had a good potential performance on pollutant 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.

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Declarations

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References

Albers CN, Ellegaard-Jensen L, Harder CB, Knudsen BE, Ekelund F, Aamand J (2015) Groundwater chemistry determines the
prokaryotic community structure of watersheds sand filters[J]. Environ Sci Technol 49(2):839–846
Anwar Sadat M, Guan Y, Zhang D, Shao G, Cheng X, Yang Y (2020) The associations between river health and water resources management lead to the assessment of river state. Ecol Indic 109:105814
Breštic M, Zivkac M (2013) PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. Molecular stress physiology of plants. https://doi.org/10.1007/978-81-322-0807-5_4
Cappelletti M, Presentato A, Placenza E, Ferrinciari A, Turner RJ, Zannoni D (2020) Biotechnology of Rhodococcus for the production of valuable compounds. Appl Microbiol Biotechnol 104(20):8567–8594
Chouari R, Le Paslier D, Daegelen P, Ginestet P, Weissenbach J, Sghir A (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digestor. Environ Microbiol 7(8):1104–1115
Fatahi-Bafghi M (2019) Antibiotic resistance genes in the Actinobacteria phylum. Eur J Clin Microbiol Infect Dis 38(9):1599–1624
Frankenbach S, SeroDio J (2017) One pulse, one light curve: fast characterization of the light response of microphotobenthos biofilms using chlorophyll fluorescence. Limnology and Oceanography: Methods
Giripunje MD, Fulke AB, Meshram PU (2015) Remediation techniques for heavy-metals contamination in lakes: a mini-review. Clean Soil Air Water 43(9):1350–1354
Gao C, Cui Y, Dong B, Luo Y, Liu F, Zhao S, Wu H (2017) Test study of the optimal design for hydraulic performance and treatment performance of free water surface flow constructed wetland. Bioresour Technol 238:461–471
Guo Z, Kang Y, Hu Z, Liang S, Xie H, Ngo HH, Zhang J (2020) Removal pathways of benzofluoranthene in a constructed wetland amended with metallic ions embedded carbon. Bioresour Technol 311:123481
Hassan SS, Anjum K, Abbas SQ, Akhter N, Shagufa BI, Shah SA, Tasneem U (2017) Emerging biopharmaceuticals from marine actinobacteria. Environ Toxicol Pharmacol 49:34–47
Hirsch RM, Moyer D, Archfield SA (2010) Weighted Regressions on Time, Discharge, and Season (WRTDS), with an application to Chesapeake Bay river inputs. J Am Water Resour Assoc 46(5):857–889
Hou X, Liu G, Jiang G (2018) Metabolism of typical halogenated organic pollutants in plants. Sci Sin Chim 48(10):1236–1246
Huang X, Yang X, Zhu J, Yu J (2020) Microbial interspecific interaction and nitrogen metabolism pathway for the treatment of organic pollutants in plants. Sci Sin Chim 48(10):1236–1246
Jahn C, Cui Y, Zhang D, Shao G, Cheng X, Yang Y (2020) The associations between river health and water resources management lead to the assessment of river state. Ecol Indic 109:105814
Kobayashi Y, Ralph TJ, Sharma P, Mitrovic SMJM, Research F (2020) Influence of historical inundation frequency on soil microbes (Cyanobacteria, Proteobacteria, Actinobacteria) in semi-arid floodplain wetlands Marine and Freshwater Research 71(5):617–625
Kralj JPJPP (2010) Relationship between photosystem II activity and CO2 fixation in leaves. Physiol Plant 86(1):180–187
Kushwaha A, Hans N, Kumar S, Rani R (2018) A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies[J]. Ecotoxicol Environ Saf 147:1035–1045
Ladislav S, El-Mufleh A, Gérente C, Chazarenc F, Andrs Y, Béchet B (2011) Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban stormwater runoff. Water Air Soil Pollut 223(2):877–888
Li D, Zheng B, Liu Y, Chu Z, He Y, Huang M (2018) Use of multiple water surface flow constructed wetlands for non-point source water pollution control. Appl Microbiol Biotechnol 102(13):5355–5368
Li X, Li Y, Li Y, Wu J (2020a) Myriophyllum elatinoides growth and rhizosphere bacterial community structure under different nitrogen concentrations in swine wastewater. Bioresour Technol 301:122776
Li X, Wu S, Yang C, Zeng G (2020b) Microalgal and duckweed based constructed wetlands for swine wastewater treatment: a review. Bioresour Technol 318:123858
Li X, Zhang L, Yang Z, Wang P, Yan Y, Ran J (2020c) Adsorption materials for volatile organic compounds (VOCs) and the key factors for VOCs adsorption process: a review. Sep Purif Technol 235:116213
Li JJ, Dong B, Guo CQ, Liu FP, Brown L, Li Q (2016) Variations of effective volume and removal rate under different water levels of constructed wetland. Ecol Eng 95:652–664
Long Y, Wu S, Xiao Y, Cui P, Zhou H (2018) VOCs reduction and inhibition mechanisms of using active carbon filler in bituminous materials. J Clean Prod 181:784–793
Lu G, Wang B, Zhang C, Li S, Wen J, Lu G, Zhu C, Zhou Y (2018) Heavy metals contamination and accumulation in submerged macrophytes in an urban river in China. Int J Phytoremediation 20(8):839–846
Ma XL, Yao SJ (2011) Construction of mixed hydrogen-producing bacteria in anaerobic activated sludge. J Chem Eng High Educ, 25(1):6
Marc S, Herrmann R, Barbara B, Bertram K, Peter G (2013) Integrated monitoring of particle associated transport of PAHs in contrasting catchments[J]. Environ Pollut 172:155–162
Man Y, Wang J, Tam NF, Wan X, Huang W, Zheng Y, Tang J, Tao R, Yang Y (2020) Responses of rhizosphere and bulk substrate microbiome to wastewater-borne sulfonamides in constructed wetlands with different plant species. Sci Total Environ 706:135955
Mandal B, Das SC, Mandal LNJP, Soil (1992) Effect of growth and subsequent decomposition of cyano bacteria on the transformation of phosphorus in submerged soils. Plant Soil 143(2):289–297
Mathon B, Coquery M, Miege C, Vandycke A, Choubert JM (2019) Influence of water depth and season on the photodegradation of micropollutants in a free-water surface constructed wetland receiving treated wastewater. Chemosphere 235:260–270
Milošedzka A, Muszynski A (2015) Population dynamics of filamentous bacteria identified in Polish full-scale wastewater treatment plants with nutrients removal. Water Sci Technol 71(5):675–684
Mun H, Townley HE (2021) Nanoencapsulation of plant volatile organic compounds to improve their biological activities. Planta Med 87(3):236–251
Navarro-Ortega A, Tauler R, Lacorte S, Barcelo D (2010) Occurrence and transport of PAHs, pesticides and alkylphenols in sediment samples along the Ebro River Basin. J Hydrol 383(1–2):5–17
Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Marmonier P (2012) Sedimentary context controls the influence of ecosystem engineering by bioturbators on microbial processes in river sediments. Oikos 121(7):1134–1144
Nilsson C, Polvi LE, Gardeström J, Hasselquist EM, Lind L, Sarneel JM (2015) Riparian and in-stream restoration of boreal streams and rivers: success or failure. Ecosystems 8(5):753–764
Nubel U, Bateson MM, Vanderkelen V, Wieland A, Kuhl K, Ward DM (2002) Microscopic examination of distribution and phenotypic properties of phylogenetically diverse Chloroflexaceae-related
