Luciana Schultze-Nobre¹Arndt Wiessner^{1*}Cindy Bartsch¹Heidrun Paschke²Alexandros I. Stefanakis^{3,4}Lara A. Aylward^{1,5}Peter Kusch^{1†}

Research Article

Removal of dimethylphenols and ammonium in laboratory-scale horizontal subsurface flow constructed wetlands

Phenolic compounds in industrial wastewaters are toxic pollutants and pose a threat to public health and ecosystems. More recently, focus is being directed toward combining the treatment of these compounds with a cost-effective and environmentally sound technology. The removal efficiency of dimethylphenol and ammonium nitrogen was studied, for the first time, in three different laboratory-scale horizontal subsurface flow constructed wetlands planted with *Juncus effusus*. Two of the wetlands used were filled with gravel. One of these was planted and the second left without vegetation. The third wetland was a hydroponic system. It was found that the removal efficiencies of dimethylphenol was dependent on the inflow loading of the contaminant and was higher in the planted systems. Both planted systems yielded 99% removal efficiency up to loads of 240 mg/d, compared to only 73% for the unplanted constructed wetland. Factors and processes such as redox dynamics, methanogenesis, reduction of ammonium and low nitrate and nitrite concentrations imply simultaneous aerobic and anaerobic dimethylphenol transformations. A significant surplus of organic carbon was detected in the planted wetlands, which may originate from intermediates of the dimethylphenol transformation processes and/or organic plant root exudates. The present study demonstrates that horizontal subsurface flow constructed wetlands are a promising alternative system for the treatment of effluents contaminated with dimethylphenol isomers.

Keywords: Ammonium / Constructed wetlands / Dimethylphenol / *Juncus effusus* / Methane

Received: October 7, 2016; accepted: October 5, 2017

DOI: 10.1002/elsc.201600213

¹Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

²Department of Analytical Chemistry, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

³Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

⁴Bauer Resources GmbH, Schrobenhausen, Germany

⁵University of the Witwatersrand, School of Chemical and Metallurgical Engineering, Johannesburg, South Africa

1 Introduction

Phenolic compounds generated during industrial and agricultural processes are hazardous wastewater contaminants due to their toxicity and resistance to biological degradation [1–6]. Due to limited resources of petroleum oil and natural gas and various economic constraints, a partial substitution of the petrochemicals by the carbochemicals is expected [7, 8]. Effluents from the carbochemical industry, such as coke wastewater, contain considerable amounts of phenolic compounds and other inorganic species, including ammonium [9]. The established physicochemical techniques for the treatment of such wastewaters are neither environmentally sound nor cost effective due

to high prices of appropriate sorbents and catalysts and their expensive regeneration processes.

Constructed wetlands (CWs) may be considered an effective treatment alternative for various wastewaters and sludges [5, 10–12], with studies already presenting promising results for the degradation of phenol in these systems. For example effluent from olive mills [13, 14], wineries [6, 15], coffee processing [16, 17], cork processing [18] and the pulp and paper industry [19, 20]. However, literature documenting the treatment of petrochemical and, particularly, carbochemical industrial effluents using CWs is limited [21–23].

Different classes of CWs, as well as classical sand filters, have been investigated for wastewater treatment [5]. Rossmann

Correspondence: Luciana Schultze-Nobre (luciana.schultze-nobre@ufz.de), Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

Abbreviations: DMP, dimethylphenol; G-CW, unplanted gravel constructed wetland; HSSF, horizontal subsurface flow; PG-CW, planted gravel constructed wetland; P-CW, hydroponic constructed wetland; SOC, surplus organic carbon; TOC, total organic carbon

*Additional correspondence: Dr. Arndt Wiessner (arndt.wiessner@ufz.de), Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, 04318 Leipzig, Germany

[†]Peter Kusch passed away on the 14th of October 2014.

et al. [17] found that artificial aeration and vegetation led to an improvement of the pollutant removal efficiency with regard to nitrogen, phosphorus and phenolic compounds. It was hypothesized that plants play an important role as oxygen suppliers and habitats for the development of microbial communities in the root zone – both of which promote biodegradation processes. The results of these experiments also indicated that phenol uptake by the plants was of minor importance as previously suggested by Kurzbaum et al. [24]. Phenol removal was mainly attributed to the action of bacteria in the biofilms attached to the gravel and roots.

Generally, most of the available studies on phenol degradation in CWs report results for total phenol removal (the sum of all phenolic compounds) but there are minimal studies investigating the fate of specific phenolic compounds, such as Bisphenol A, *m*-cresol and chlorinated phenols [20, 23, 25]. In particular, there are only a few studies focusing on the elimination of dimethylphenols (DMPs) from complex ecosystems and groundwater aquifers [1], in single plant hydroponic sand-bed systems [6, 26] and in a laboratory-scale planted fixed bed reactor (PFBR) [27]. Specifically, experiments in a PFBR system planted with *Juncus effusus* showed that a stepwise increase in the inflow DMP concentration from 20 mg/L to 100 mg/L caused a decrease in total DMP removal efficiency. The highest removal efficiencies were observed for the 3,4- and 3,5-DMP isomers and the lowest for 2,6-DMP [27]. Intermediates resulting from the DMP degradation (such as dimethylbenzenediols and ketoacidic acid carboxylates) and oxidative coupling products (such as tetramethyl-[1,1'-biphenyl] diols and tetramethyl diphenylether monools) were found in small amounts [28]. The abiotic sorption potential of the roots of *Juncus effusus* toward different phenolic compounds, including DMPs and polycyclic aromatic hydrocarbons, was also studied [29]. Overall, the PFBR reactors proved to be efficient at the laboratory-scale, but such systems are not considered particularly suitable for scale-up to industrial sized operations.

A review of the available literature has provided direction for future research on DMP degradation in CW systems. Despite some knowledge about DMP transformation in a wetland environment, further studies investigating the fate of DMPs in CWs under typical plug-flow conditions are lacking. Hence, additional information is required regarding the influence of different loading rates on DMP transformation processes. Related to this is the effect of DMP loading on plant growth. Phenol toxicity to plants has been partially discussed in literature [4, 30, 31], but results of specific studies on the environmental cytotoxicity of DMPs is absent. Finally, the influence of different wetland designs on the fate of DMPs should be further investigated.

In the present study, long-term experiments were conducted using three laboratory-scale horizontal subsurface flow (HSSF) CWs of the same dimensions but different internal designs. Focus was placed on measuring the removal efficiencies of various DMP isomers and ammonium, as well as on evaluating the effect of loading rate on: the spatial distribution of concentrations, plant health, the balance of organic carbon and related DMP transformation processes.

2 Materials and methods

2.1 Experimental design and conditions

The model experiments were performed in three laboratory-scale HSSF CWs (1 m length $\times$ 0.15 m width $\times$ 0.35 m depth, surface of 0.15 m²) from December 2012 to August 2013. Two units were filled with fine gravel (mass 54 kg, average particle size 2–4 mm and porosity 38.3%). One of these was planted with common rush (*Juncus effusus*) and designated the planted gravel bed (PG-CW), while the other was left without vegetation and named the unplanted gravel bed (G-CW). The water level was maintained at 1 cm below the gravel surface. The third system was designed as a hydroponic system (P-CW), also containing *Juncus effusus*. Before commencing the experiments, the two planted systems were allowed to establish for a period of 3 months without any external nutrient source or contaminant load.

Each wetland was fed continuously with artificial wastewater at an inflow rate of 6 L/d; corresponding to a hydraulic loading rate of 40 L/m²/d. The theoretical hydraulic retention times (HRTs) were 2.5 days in the gravel systems and 4.3 days in the hydroponic system. The artificial wastewater was prepared in accordance with standard artificial wastewater protocols (DIN-38412-T24, 1981), but the sulfate load was reduced to limit microbial dissimilatory sulfate reduction processes. The artificial wastewater contained (in mg/L): 79.1 NH₄Cl, 24.7 K₂HPO₄, 4.7 NaCl, 2.7 CaCl₂·2 H₂O, 1.1 MgCl₂·6 H₂O, 0.6 Na₂SO₄ (dissolved in deionized water) and 252.0 NaHCO₃. The resultant inflow composition (in mg/L) was: 20.7 ammonium-N, 4.4 phosphate-P, 0.2 sulphate-S and 36.0 inorganic carbon. The synthetic wastewater contained an equimolar mixture of 2,6-, 3,4- and 3,5-DMP isomers (Sigma-Aldrich, Munich, Germany) as the only additional organic carbon source. Furthermore, the artificial wastewater contained 1 mL/L of trace mineral solution comprising (in g/L): 1.0 EDTA-Na, 1.0 FeSO₄·7 H₂O, 0.8 MnCl₂·4 H₂O, 1.7 CoCl₂·5 H₂O, 0.7 CaCl₂·6 H₂O, 1.0 ZnCl₂, 1.5 CuCl₂·5 H₂O, 0.3 NiCl₂·6 H₂O, 0.1 H₃BO₃, 0.1 Na₂MoO₄·2 H₂O and 0.02 Na₂SeO₃·5 H₂O.

The CWs were operated under controlled environmental conditions in a greenhouse. The temperature was set at 22°C from 6 am to 9 pm and at 16°C overnight. An artificial light source (Master SON-PIA 400W, Phillips, Belgium) was set to turn on when the natural light fell below 60klx during the day-time. The experiments were carried out in five phases with the following DMP inflow loads: Phase A (0–36 days) with 120 mg/d (20 mg/L and 15.7 mgC/L); Phase B (37–156 days) with 240 mg/d (40 mg/L and 31.5 mgC/L); Phase C (156–211 days) with 600 mg/d (100 mg/L and 78.6 mgC/L); Phase D (212–240 days) with 240 mg/d (40 mg/L and 31.5 mgC/L); Phase E (241–280 days) with 120 mg/d (20 mg/L and 15.7 mgC/L).

2.2 Water sampling and analysis

Water samples were taken from the inflow, outflow and at distances of 0.13, 0.25, 0.50, and 0.75 m from the inflow point. All samples were removed from a depth of 17 cm below the

gravel surface (mid-depth in the bed) using steel lances (3.5 mm inner diameter) and a peristaltic pump (BVP_Z, ISMATEC SA, Switzerland). The water was pumped through a flow cell, and the redox potential (SenTix ORP Pt/Ag⁺/AgCl/Cl[−], WTW, Germany), pH (pH 539, WTW, Germany) and temperature (Checktemp1, Hanna Instruments, Germany) were recorded. To measure the dissolved oxygen concentration, an optical trace oxygen mini sensor (FTC-TOS7-PSt3, PreSens GmbH, Germany) and a Fibox-3-Trace single channel fiber-optic trace oxygen meter were used (PreSens GmbH, Germany).

The DMP concentrations were analyzed by means of an HPLC prominence line (Shimadzu, Japan), equipped with LC Solution Postrun Analysis software, a degasser DGU-20A3, a binary gradient pump LC-20AB, an autosampler SIL-20A, a column oven CTO-20AC and a UV-detection system SPD-M20A. The column and security guard used were both Poroshell 120 EC-C18 2.7 μ m 3.0 $\times$ 150 mm units (Agilent Technologies, USA). The column was operated at a constant temperature of 50°C. The mobile phase consisted of 0.1% (v/v) formic acid and 0.1% (v/v) acetonitrile in water (Eluent A), and acetonitrile (Eluent B) and the gradient program was selected to be 20% B to 40% B (45 min). The flow rate was 0.2 mL/min and the injection volume for all samples was 30 μ L. Spectra were recorded from 190 to 600 nm. DMP was detected at 278 nm and identified by comparison with known standards.

Merck quick tests (NH₄⁺: 1.00683.0001, NO₃[−]: 1.09713.0001, NO₂[−]: 1.14776.0001) and the Spectroquant Nova 60 photometer (Merck KGaA, Germany) were applied for the determination of the ammonium, nitrate and nitrite concentrations. The concentration of total organic carbon (TOC) was measured using a TOC-analyzer VC_{SN} (Shimadzu, Japan). Methane (CH₄) was analyzed in duplicate by means of a headspace GC-FID 6890 (Agilent Technologies, USA) using a CP-Sil 8CD column with pure helium as a carrier gas. All computations in this study were performed using OriginPro[®] 9G software (OriginLab Corporation, USA).

The *Juncus effusus* stalk density was determined weekly. The planted CWs were divided into four sections; namely 0–0.25 m, 0.25–0.5 m, 0.5–0.75 m, and 0.75–1 m and the mean vegetation density in each section was calculated. For the evaluation of the mass of water lost and the calculation of contaminant loads, water balances on the inflow and outflow were performed twice per week.

3 Results

3.1 Overall DMP removal efficiency

For the calculation of contaminant loading, a linear increase in water loss along the flow path was assumed due to the uniform spatial distribution of the plants. Figure 1 shows the inflow and outflow DMP loading over the duration of the experiment. Higher removal efficiencies were observed in the PG-CW compared to the G-CW during all phases. Almost complete degradation and transformation was observed in the planted systems for loads up to 240 mg/d (phases A, B, D and E), while an average

Figure 1. DMP load in the planted gravel (PG-CW), unplanted gravel (G-CW) and hydroponic (P-CW) wetland systems.

of 73% DMP removal was found in the G-CW. The increase in DMP load up to 600 mg/d (phase C) reduced the removal efficiency in all CWs. During this phase, an average of 82% DMP removal was observed in both planted wetlands, whilst only 51% removal was obtained in the G-CW.

3.2 The removal efficiency of 2,5-, 3,4-, and 3,5-DMP

Figure 2 illustrates the removal dynamics of the single DMP isomers in each of the three CWs. Again, the planted systems showed higher removal efficiencies for all isomers, with close to 100% degradation during phases A, B, D and E. The increased inflow loading during phase C reduced the removal efficiency of all three systems.

3,4-DMP showed the highest removal efficiency in all three phases and across all three systems. Almost complete transformation was achieved with the exception of phase C, where an average removal efficiency of 91, 92, and 64% in the PG-CW, P-CW and G-CW systems, respectively, was calculated. An average of 75–77% removal of 3,5- and 2,6-DMP was observed during phase C in the planted CWs, but only 41% in the unplanted systems.

3.3 Spatial distribution of DMP

The removal rate of the DMPs varies along the length of each wetland system, especially during phase C, as shown in Fig. 3. A high removal rate was observed within the first (0–0.25 m) and last (0.75–1.0 m) sections of the planted gravel systems (Fig. 3A and B), whereas a noticeable removal efficiency was also found in the middle sections (0.25–0.75 m) of the P-CW (Fig. 3B). The most significant DMP removal was found only in the inflow section of the G-CW system (Fig. 3C).

Figure 2. Inflow DMP and outflow 2,6-, 3,4- and 3,5-DMP loads in the (A) planted gravel (PG-CW) (B) hydroponic and (C) unplanted gravel (G-CW) systems.

3.4 Plant biomass density and physicochemical parameters

The mean stalk density in the planted wetlands, as presented in Fig. 4A, was determined as the average of the mean densities calculated weekly in each of the four sections along the wetland length, as it was found that the spatial distribution of the plants was approximately uniform throughout. The mean

Figure 3. Inlet, outlet and internal DMP load in the (A) planted gravel (PG-CW), (B) hydroponic (P-CW) and (C) unplanted gravel (G-CW) systems.

specific stalk densities of the plant stocks in the two planted CWs are given in Fig. 4A. The initially well-developed plant mat density (7000 stalks/m²) in the P-CW decreased moderately until the middle of phase B and then drastically to just over 1000 stalks/m² by the end of phase C, where after it remained constant until the end of the experimental period. In contrast, the low plant mat density in the PG-CW at the start of the experiment increased rapidly up to the density range in the P-CW by

Figure 4. (A) Mean stalk density (B) mean redox potential (Eh) and (C) mean pH for the planted gravel (PG-CW) and hydroponic (P-CW) and unplanted gravel (G-CW) systems.

mid-way through phase B. Plant growth was then constant until the end of phase C before increasing again to match the P-CW starting density of 7000 stalks/m² by midway through phase E.

Despite the differing initial redox conditions of the CW pore waters, as shown in Fig. 4B, similar Eh values in the range of 180–260 mV were measured in all wetlands at the end of phase C. The potentials proceeded to increase almost simultaneously to values around 400 mV at the end of the experiment (Fig. 4B).

The pH of the artificial wastewater was measured to be 7.5. Generally, the pH of the wastewater decreased immediately after entry into either of the planted CWs to within the range of 6.6–6.8, as indicated in Fig. 4C). On the other hand, the pH levels in the unplanted system remained close to the initial value (7.25–7.65) until the end of the experiment. During the phase of high DMP load (phase C), the pH in the P-CW increased more significantly as compared to the PG-CW (Fig. 4C). The dissolved oxygen was consistently measured to be in the range of 0.5–1.0 mg/L in all CWs (data not shown).

3.5 Organic carbon and methane production

Higher loads of total organic carbon (TOC) were found in the outflow of the three CWs compared to the inflow, as shown in Fig. 5. The additional TOC was characterized as untransformed DMP and traces of methane carbon. After the determination of the contribution of untransformed DMP and methane to the outlet TOC load, the balance of organic carbon which was still unaccounted for (undefined) was termed surplus organic carbon (SOC). Figures 5A, B and C show the progression of both TOC and DMP carbon in the outflow over the duration of the experiment. Figures 5D, E and F illustrate the quantities of methane measured at three locations in the CW systems.

In the G-CW system, nearly 70% of the total TOC (32.661 mg) in the outflow over the experimental period was determined to be the remains of DMP and generated methane carbon (although only in trace amounts as seen in Fig. 5F) and 30% could be characterized as SOC.

In contrast, 74% of the TOC (24.9 mg) detected in the PG-CW system outlet was SOC with the balance being untransformed DMP (25%) and methane carbon (1%). The P-CW system displayed similar results: the outlet TOC (27.3 mg) was made up of 79% SOC, 18% untransformed DMP and 3% methane-carbon. Evidently, SOC in the outflow of both planted CWs is of importance in experimental phases A, B, D and E, during which inflow DMP loads of up to 240 mg/d were applied and methane was only measured in trace amounts (Fig. 5A, B, D and E).

3.6 Ammonium removal efficiency

The dynamics of ammonium removal within the three CWs over the experimental period is represented by the inflow and outflow loads in Fig. 6. In excess of 90% of the input ammonium was removed from both planted CWs during phases A and B (low DMP inflow load). In contrast, only approximately 28% was eliminated in the G-CW during these phases. The increase in inflow DMP load to its highest value (phase C) reduced the efficiency of ammonium removal to approximately 76% in the PG-CW wetland and a largely terminated removal in the P-CW. Continuing into phases D and E, ammonium removal remained relatively stable between 70 and 80% in the PG-CW system, but fluctuated noticeably in the P-CW and unplanted wetlands. Ammonium removal in these two wetlands stabilized during phase E; remaining close to 34%.

Figure 5. Outflow loads of total organic carbon (TOC), DMP-carbon and methane-carbon in the (A & D) planted gravel (PG-CW) (B & E) hydroponic (P-CW) and (C & F) unplanted gravel (G-CW) systems. Inflow TOC loads: 94.6 mg C/d (Phases A & E), 188.6 mg C/d (Phases B & D), 471.5 mg C/d (Phase C)

Figure 6. Ammonium load in the planted gravel (PG-CW), unplanted gravel (G-CW) and hydroponic (P-CW) wetland systems.

4 Discussion

This study is the first to show HSSF CW's are efficient in the removal of DMPs from artificial industrial wastewater. As demonstrated in Fig. 1, DMP removal depends on the inflow loading and the CW characteristics. In general, the PG-CW simultaneously removed up to 3.3 g DMP/m²/d and 0.65 g NH₄⁺-N/m²/d under the highest DMP load (phase C), for influent loads of 4 g DMP/m²/d and 0.8 g NH₄⁺-N/m²/d. These removal efficiencies confirm the higher removal potential for phenol compounds found in another study using HSSF CWs planted with *Phragmites australis* [23]. The removal efficiency of both planted CWs was also significantly higher compared to the unplanted system, as also found in other studies [23], indicating the importance of the plants in contaminant removal, irrespective of the presence of a solid matrix (Fig. 1). In addition, the results indicate that DMP inflow loads exceeding 240 mg/d and 40 mg/L are above the limit for complete DMP removal. Generally, the load limits seemed to be within the ranges reported for other phenolic compounds using *Eichornia crassipes* [4].

3,4-DMP was removed at the highest rate in all three systems. Shingler et al. [32] described the *meta*-cleavage pathway for (substituted) catechol as one of the major routes for the microbial degradation of aromatic compounds such as phenol, cresol and 3,4-DMP. Experiments performed in a PFBR showed the same elimination course and provided indications of a possible *meta*-cleavage pathway [26,28].

The results obtained during this study confirm the importance of plants in phenolic compound removal processes, which is in line with previous findings [4, 5, 23, 26, 33, 34]. They also verify the strong influence of inflow loads on removal efficiency [3, 14, 16, 23, 27]. Similar DMP removal dynamics were found in both planted CWs, (Fig. 1), despite the differences in the stalk densities (Fig. 4A). This finding implies not only a high potential for DMP transformation within the planted systems (independent of the density of the above-ground biomass) but also the beneficial role of the roots, the radial oxygen loss and the associated biofilms, as also mentioned elsewhere [24].

It is known that wetland plant species can, to some extent, assimilate phenolic compounds in their roots via various processes such as phytodegradation, phytostabilization, and phytoaccumulation [33, 35–38]. This has already been reported for the species *typha latifolia* and *Lolium multiflorum* Lam [17, 33, 39]. However, the exact pathway and extent of plant uptake are not yet clear. Overall, it is believed that the amount of phenolic compounds that plants assimilate to their biomass in CW systems is small [40], although could be higher compared to other wastewater pollutants such as nitrogen or phosphorus [41].

The gradual decrease in plant stalk density in the P-CW and simultaneous increase in the PG-CW implies that plants were stressed when plant roots were directly exposed to the DMPs in the water column without the presence of gravel (Fig. 4A). The related toxicity may be caused not only by the inflow DMP load but also by the generation of intermediates during phenol transformation, such as catechol, hydroquinone and other organics [3, 6, 29]. The negative effects of high DMP concentrations on the growth of *Juncus effusus* and the elimination efficiency have already been reported [27–29]. However, the gradual increase in stalk density in the PG-CW clearly indicates a limited toxicity effect in this CW design. Generally, plant species used in wetland systems have proven tolerant to phenol. Nevertheless, the toxicity of phenolic compounds to different helophytes, especially at high phenol loading rates, requires further investigation.

The high outflow loads of SOC, in particular in the PG-CW, may be produced not only by DMP transformation products but also by organic exudates released by the plants as a response to toxicity stress. SOC formation was found to be more intensive in the P-CW system, implying that the plants were more stressed in the hydroponic system as compared to the PG-CW. In general, SOC is considered a potential source of organic carbon for heterotrophic microbial processes within the rhizosphere; thereby stimulating denitrification, sulfate reduction or even undesirable methanogenesis. The combination of plants with a solid substrate medium seems to act beneficially against the intoxication of the plants and microorganisms at high DMP loads. The low initial plant density in the PG-CW system increased after the increase in DMP load (phase B), while the much higher initial

density in the P-CW system decreased (Fig. 4A). This confirms previous findings by Kurzbaum et al. [24].

High DMP loads seem to affect not only plant growth but also ammonium removal in the planted CWs (Figs. 4A & 6). The high initial density of the plant stock in the P-CW system decreased drastically as the DMP load increased and the highest DMP load (phase C) halted the growth of the PG-CW stock. At the same time, the ammonium outflow load increased in both planted systems, but was more drastically in the P-CW system and outlet loads were not stable during phase C and thereafter (Fig. 6). As mentioned above, DMP degradation and ammonium oxidation (nitrification) are mainly aerobic processes. Assuming, based on current experience, that biodegradation is the main removal process for these compounds, it appears that DMP and ammonium are competitors regarding the consumption of the available oxygen by the relevant microbes. At both high and low DMP loading rates, there seems to be a preference for DMP degradation, given that the ammonium removal rates were always lower than the DMP removal rates. This could mean that the composition of the microbial community is dominated by phenol-degrading bacteria [23], which overcome nitrifying bacteria in the competition for oxygen consumption. This is also supported by the results of the unplanted bed, where limited available oxygen resulted in reduced removal rates for both DMP (Fig. 1) and ammonium (Fig. 6), but still DMP removal was higher than that of ammonium. It is characteristic that under the highest DMP load (phase C), the planted CWs removed more than 3 g DMP/m²/d compared to only 0.6 g NH₄⁺-N/m²/d (respective values for the unplanted bed: 2 g DMP/m²/d and 0 g NH₄⁺-N/m²/d).

The high plant density, the high and increasing redox values and the different DMP-isomer's removal rates indicate the presence of mainly aerobic microbial transformation processes and secondary the uptake and adsorption of the phenols by plant matter and substrate media [5, 17, 27, 29]. The necessary oxygen may be supplied either by the plant roots, the oxygen dissolved in the influent saturated wastewater or by air diffusion through the unsaturated gravel zone into the upper pore water of the wetland. On the other hand, the relatively high methane enrichment with a maximum of 15% (Fig. 5), particularly at the front section of the wetland, as well as the absence of nitrate and nitrite (data not shown) despite the high rate of ammonium transformation, implies the presence of anaerobic conditions and the anoxic processes of carbon and nitrogen transformation. Therefore, results indicate the simultaneous presence of both aerobic and anaerobic sites within the wetland body, which promote the respective transformation processes for both DMPs and ammonium.

The inflow section of all CWs was found to be the most important for DMP removal, as presented in Fig. 3. This finding has also been found elsewhere [23] and suggests that inflow conditions (higher oxygen availability, higher DMP concentrations and higher microbial biodiversity) favor specific transformation processes, for example aerobic transformation. At the same time, methane presence in the inflow section (Fig. 5) indicates that anaerobic microbial processes also take place. Figure 3 shows that the majority of DMP removal takes place in the first section of the wetland length. This may be related with results of previous studies reporting that, in HSSF CWs, microbial biomass density (and respective microbial pollutant transformations/removals) are concentrated in the front section of the wetland and

decreases with distance from the inlet [42, 43]. On the other hand, the possible toxicity of high inflow DMP loads could favor the chemical and/or physicochemical transformation processes and limit biodegradation.

The absence of the substrate medium and the higher HRT in the P-CW did not significantly affect DMP removal (Fig. 1). The hydraulic residence time in the P-CW was higher, for the same loading rate, due to the absence of gravel and the higher effective volume of the system. In general, nearly similar removal dynamics were found in the two planted CWs (with and without gravel) for the whole experimental operation period (Fig. 1). However, when comparing the PG-CW and G-CW, the spatial distribution of the removal along the flow path is different, since the removal rate in the PG-CW was higher (Fig. 3). Apparently, the presence of plant roots within the gravel provides an increased surface area for the development of the biofilm community; thereby increasing microbial community density and resulting in faster degradation, particularly in the inlet section of the wetland [23, 33].

When assessing the dynamics of nitrogen removal in CWs, it is generally agreed that plants play an important role in nitrogen removal, both as oxygen supplier for nitrification and nitrogen consumer to provide for their growth needs [5, 14, 35, 42, 44]. Apart from some fluctuations under high DMP load conditions, ammonium removal in the G-CW system was limited (Fig. 6) and could be attributed to adsorption onto the gravel media. In contrast, almost complete ammonium removal was observed in the planted systems during phases A and B with low DMP load (Fig. 6). Apart from nitrogen uptake by plants, the beneficial role of the plants lies in the advantageous micro-gradient conditions in the near-root environment (oxic/anoxic micro-zones for nitrification/denitrification). Accordingly, the radial oxygen loss by the helophytes and the bioavailability of organic substances are of importance [45–47]. However, the results of this study suggest a much higher sensitivity of the P-CW to disturbances, such as inhibition of nitrogen transformation by high DMP load, compared to the PG-CW system. Thus, the adjustment to the maximum DMP load caused a rapid decline in nitrogen transformation in the P-CW system and also reduced the efficiency after the re-adjustment (Fig. 6).

5 Conclusions

Both planted and unplanted HSSF CWs are, in principle, capable of removing DMP (up to 3.3 g DMPs/m²/d) from wastewater. The removal efficiency depends on the DMP loading. Highly efficient DMP removal was determined for inflow loads up to 240 mg/d (40 mg/L), whereas loads of 600 mg/d (100 mg/L) resulted in a decrease in treatment efficiency. Removal efficiency was also found to be higher in planted systems, as compared to unplanted wetlands, although the presence of a gravel bed, as well as the density of the above-ground plant biomass, did not significantly influence the efficiency of DMP removal between the two planted CWs.

Higher amounts of SOC were detected in the P-CW; suggesting incomplete DMP transformation and/or a more intensive release of organic substances from the plant roots as a response to possible toxic stress. Both aerobic and anaerobic processes

should be responsible for carbon and nitrogen transformation and methane generation, which is of greatest importance in the inflow sections of both planted CWs. Ammonium removal was found to be highly efficient in the planted CW and insufficient in the unplanted counter-part. Further research is necessary to evaluate not only the toxicity of the DMP isomers to the plants, the respective carbon and nitrogen transformations and the importance of the root/solid/biofilm complexity, but also the limits of possible inflow loads as well as the nature and the dynamics of the undefined SOC.

Practical application

Engineered systems designed to harness natural decontamination pathways under controlled conditions are described at the laboratory-scale for the treatment of phenolic industrial wastewater. The long-term experiments achieved effective dimethylphenol and ammonium removal under increasing organic carbon loads. The laboratory results obtained herein are thought to be a significant basis for pilot and then industrial scale-up.

This work was supported by the Helmholtz Centre for Environmental Research – UFZ within the scope of the SAFIRA II Research Program. Further funding was provided by the Helmholtz Interdisciplinary Graduate School for Environmental Research (Higrade). The authors would like to thank Dr. Hermann Heipieper for his constructive comments and Kerstin Puschendorf and Kerstin Ethner for technical assistance. They are also grateful to the graduate students Dandan Wang, Xiaotong Ding and Leslie Sigmon for their laboratory assistance.

The authors have declared no conflict of interest.

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