4 Results and discussions

4.1 Characterization of sulphide toxicity to *J. effusus*

The results of these experimental studies show that *J. effusus*, a dominant ubiquitous emergent water plant tolerates short-term (< 25 days) roots exposition to sulphide concentrations ≤ 25 mg S^{2-} L^{-1} under hydroponic conditions. The water uptake and the relative growth rate (RGR) within the acclimatisation period and sulphide exposition are shown in Figure 17.

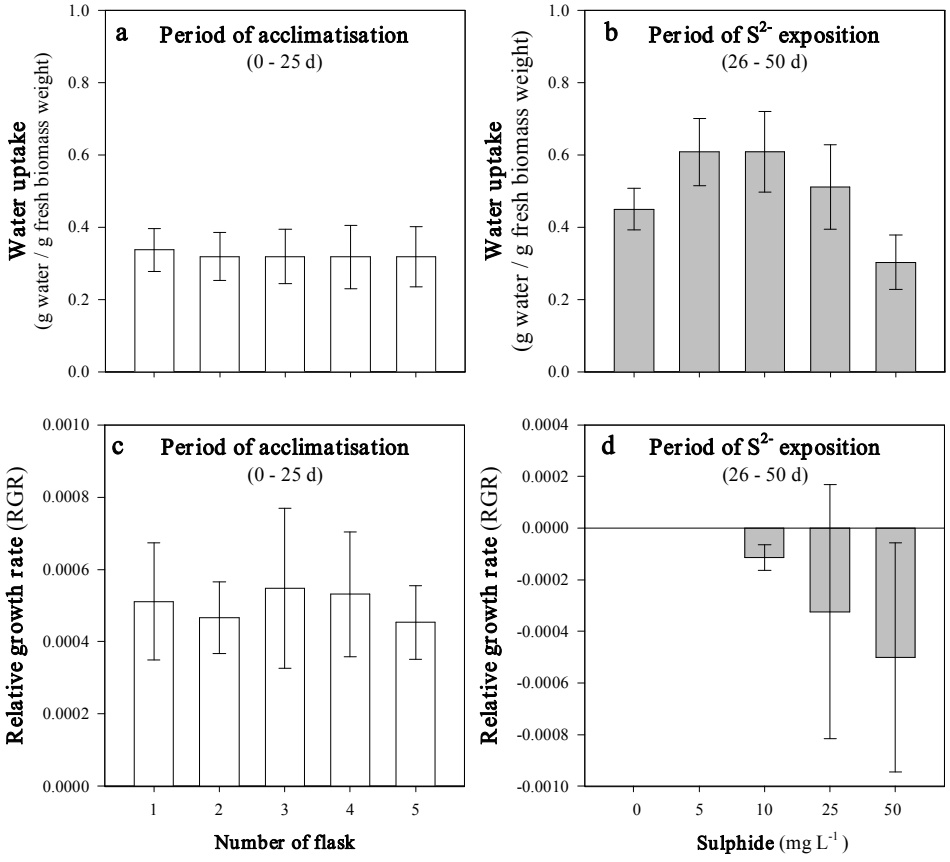

Figure 17 Mean specific water uptake (a and b) relative growth rate (c and d) of *J. effusus* depending on the exposition of sulphide in the nutrient solution. Bars shown with standard error of the mean; n = 10 (a, c) and 16 (b, d) respectively.

The chlorophyll *a* fluorescence within the acclimatisation period a) and sulphide exposition b) are shown in Figure 18.

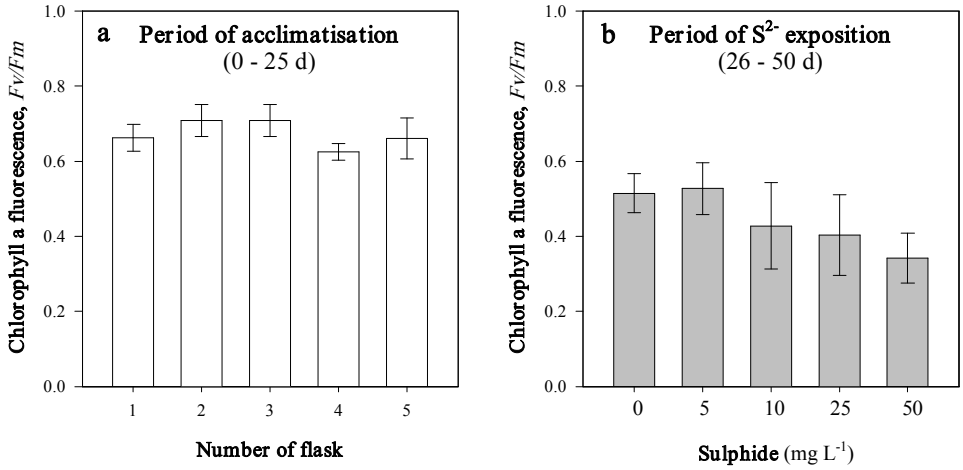

Figure 18 Chlorophyll *a* fluorescence of *J. effusus* depending on the exposition of sulphide in the nutrient solution (bars are shown with standard error of the mean; n = 10 a) and 12 b) respectively.

The water uptake during first 25 days without adding sulphide to *J. effusus* (Figure 17a), showed no differences between the flasks. In fact, no relevant reduction in RGR (Figure 17c) and chlorophyll a fluorescence (Figure 18a) could be detected. Additionally, no yellow colour on the shoots was observed. During the period of acclimation (first 25 days) the water uptake rates for *J. effusus* were similar for all five flasks in the range of 0.28 to 0.34 g water/g fresh biomass while an increase for plants exposing to 5, 10 and 25 mg S^{2-} L^{-1} was observed (Figure 17b). Sulphide concentration of 50 mg S^{2-} L-1 caused a clear reduced water uptake rate (Figure 17b). Fürtig et al., (1996) reported that plants exposed to toxic levels of phytotoxins have found to absorb less water than controls but, this was no doubt due to other factors as well as to blockage in the xylem.

The RGR during the acclimation period (first 25 days) varied in a range of 0.00045 – 0.00055 (see Figure 17c). Under sulphide exposure RGR values (Figure 17d) showed that control and 5 mg S^{2-} L^{-1} did not present growth differences, while sulphide level ≥ 10 mg S^{2-} L^{-1} reduced RGR. A minimum RGR value at applied maximum sulphide level

(50 mg S^{2-} L^{-1}) was observed.

The values of chlorophyll *a* fluorescence within the acclimation period (without sulphide) (Figure 18a) were within the range of 0.62 to 0.71 indicating optimal photochemical efficiency of the photosystem II with no significant differences between the parallel set-ups.

After 25 days of the plant exposition to sulphide the chlorophyll *a* fluorescence showed especially for sulphide concentrations ≥ 10 mg S^{2-} L^{-1} a reduced intensity (Figure 18b). Minimum chlorophyll *a* fluorescence was observed at the maximum sulphide concentration applied (50 mg L^{-1}) which also correlated with the first yellow coloration of the plants after 4 days of sulphide exposition.

Although in healthy and dark adapted shoots the maximum photosystem II efficiency (*Fv/Fm*) should amount to approximately 0.8 (Walz, 2000), the values for the acclimation period (day 0 - 25) were in a range of 0.62 - 0.71(Figure 18a). This difference could be attributed to the leaf clip adapter which is only designed to plane leafs and not to circumpolar plants such as *J. effusus*. Nevertheless the data found in this period are considered as a well healthy shoots indicator.

Inhibition of photosynthesis or of biochemical processes linked to photosynthesis by different environmental factors may affect a plant's physiological state (Krause and Weis, 1984). Sulphide is a well known inhibitor of photosynthesis (Pezeshki et al., 1988). Figure 18b showed clearly that sulphide concentration ≥ 10 mg S^{2-} L^{-1} produced inhibition of *Fv/Fm*.

At sulphide concentration of 50 mg S^{2-} L^{-1} photosystem II efficiency (*Fv/Fm*) was clearly reduced to 0.32 (Figure 18b). At this concentration shoots already became

yellow after 4 days of sulphide exposition. The yellow coloration (chlorosis) also appeared in the flasks with 25, and 10 mg S^{2-} L^{-1} but less intensive. The mechanism of chlorosis (yellow leaves) has been reported as an indicator of photoinhibition of photosynthesis in Golden Leaves (Sicher, 1998; Sicher, 1999; Takahashi, 2002).

The toxicity effect to *J. effusus* caused by the maximum sulphide concentration (50 mg S^{2-} L^{-1}) are according with some authors, who reported that sulphide concentrations in sediment pore-water > 32 mg L^{-1} have been found to induce stunted growth adventitious roots, lateral roots and buds, as well as callus formation in root and rhizomes, besides blockages in the vascular system (Armstrong et al., 1996a; Armstrong et al., 1996b).

Fürtig et al., (1996) found that *Phragmites australis* is negatively affected even at sulphide concentration in pore-water as low as 32 mg L^{-1}. Negative effects of sulphide have been found on seagrass photosynthesis (Goodman et al., 1995) and increased mortality during die-back event have also been related to sulphide exposure (Carlson et al., 1994, Holmer and Bondgaard, 2001).

Intrusion of sulphide is considered to be the main cause for rapid die-back event of T. *testudium* in Florida Bay (Borum et al., 2005).

Conclusions

Parameters such as growth, water loss and chlorophyll *a* fluorescence are useful parameter in laboratory experiments to test toxic effects of chemicals hydroponic culture.

In hydroponic culture, water uptake, RGR and chlorophyll fluorescence resulted in a good indicator parameter to estimate toxicity effects of sulphide to *J. effusus* under laboratory conditions.

Sulphide concentration above 10 mg S^{2-} L^{-1} affected biomass production and relative growth rate for *J. effusus*. The maximum applied sulphide concentration of 50 mg S^{2-} L^{-1} reduced significantly biomass production and RGR and plants became rapidly chlorosis (yellow colour).

4.2 Treatment of a model wastewater in the Planted Fixed Bed Reactor -PFBR

4.2.1 Dynamics of S-species

The data of the inflow and effluent sulphide concentration are shown in Figure 19a. The mean inflow concentration of sulphide was 1.2 ± 0.17 mg L^{-1} for the whole experimental period of 224 d with continuous supply of artificial wastewater.

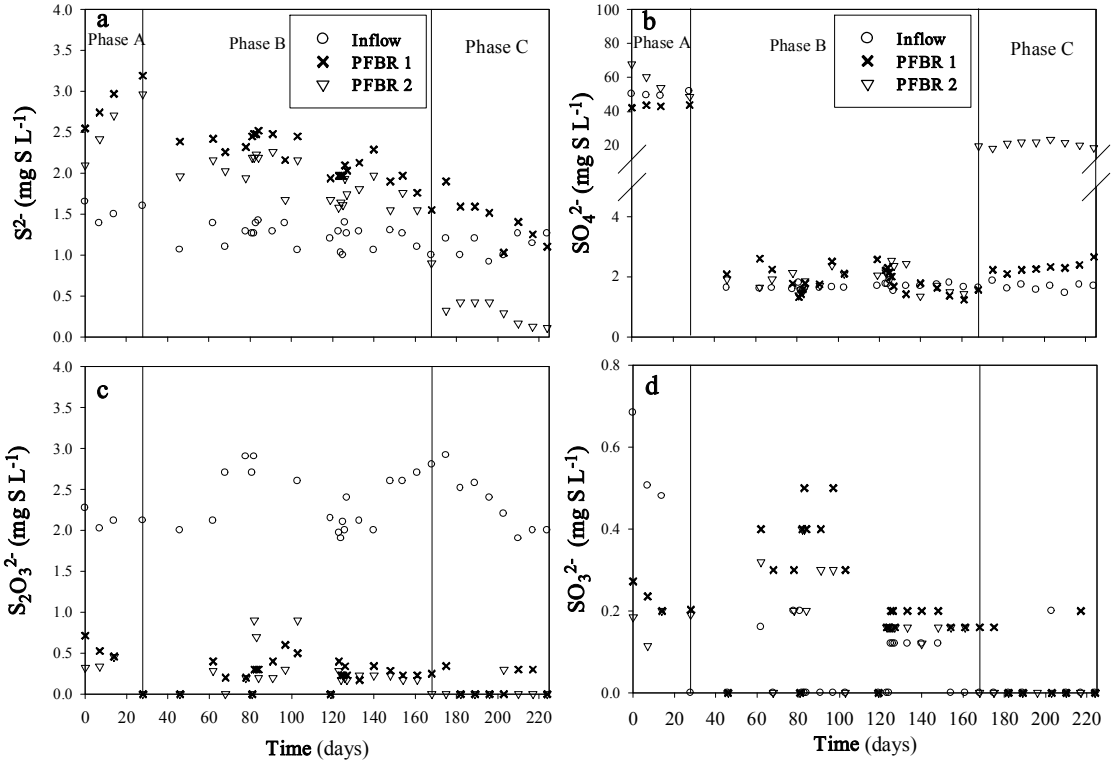

Figure 19 Sulphide a), sulphate b), thiosulphate c) and sulphite d) concentration of the inflow and outflow of the Planted Fixed Bed Reactors

Under the running condition of experimental phase A (elevated sulphate-sulphur concentration of about 50 mg L^{-1}, see Table 9) sulphide concentration was increased over the time in both planted beds to a concentration up to 2.5 times higher than that of the inflow. Due to the high inflow concentration of sulphate in this phase, sulphate

reduction is often dominating in both reactors. Sulphate reduction requires anoxic conditions which are expected to occur in the Planted Fixed Bed Reactor.

The decrease of the inflow sulphate-sulphur concentration (about 1.4 mg L^{-1} in the phases B and C, see Table 9) resulted in a slightly decrease of the sulphide concentration. Nevertheless, still in both reactors the sulphide concentrations were higher than the inflow, whereas in reactor 1 sulphide concentration was slightly higher in comparison to reactor 2 (Figure 19a).

The change of the inflow condition in phase C (doubling HRT to 10 d) resulted a decrease of the sulphide concentration in both reactors. While the sulphide concentration of the PFBR 1 in the outflow reached the level of the concentration of the inflow in this final experimental period, almost all sulphide was removed in PFBR 2. This result corresponds to an area specific removal rate of about 38 mg S $m^{-2}d^{-1}$.

Under the condition of elevated sulphate inflow concentration in phase A (of about 50 mg S L^{-1}, see Table 9) sulphate was only removed in the PFBR 1 (see Figure 19b) while sulphate outflow concentration in PFBR 2 was higher than the inflow.

In the experimental phase B the decrease of the sulphate inflow concentration (about 1.4 mg L^{-1}, see Table 4) resulted in a decrease; influent and effluent concentration of both PFBR were almost the same (Figure 19b).

By increasing the HRT to 10 d under low sulphate inflow concentration (phase C, see Table 9) the sulphate-sulphur concentration in the PFBR 2 increased surprisingly to a level of about 20 mg L^{-1} whereas in PFBR1 a very slight increase of sulphate concentration was observed.

The maximum inflow sulphite concentration reached up to 0.7 mg S L^{-1} with outflow concentration below 0.5 mg S L^{-1} (Figure 19d).

Thiosulphate sulphur (probably formed by sulphide autoxidation) with an average inflow concentration of 2.4 ± 0.3 mg S L^{-1} was well transformed in both reactors with outflow concentrations of less than 0.71 mg S L^{-1} for phases A and B (see Figure 19c). After changing the inflow parameter in phase C (increase of HRT to 10 days, see Table 9) thiosulphate concentration in the effluent stayed even below 0.3 mg S L^{-1}.

Thiosulphate concentration was reduced in all experimental phases below 1 mg L^{-1} (see Figure 19c); the other sulphur species were even only sometimes in very low concentration detectable. Nevertheless this fact does not allow making any conclusions about their role as an intermediate metabolite in the various potential sulphur transformation processes within the rhizosphere of helophytes.

During all experimental period (A, B and C; see Table 9) beside sulphide, sulphate and thiosulphate also traces of elemental sulphur and sulphite were recorded. In the case of sulphite, the values were below 0.8 mg S L^{-1} while elemental sulphur was always below the detection limit of 0.064 mg L^{-1}.

The effluent sulphide concentration trends shown in Figure 19 indicate clearly that at the hydraulic retention time (HRT) of 5 d (phase A and B) in both PFBR sulphide is produced resulting in a non removal rate (see Figure 19). The change in the inflow condition in phase C (doubling HRT to 10 d) showed a slightly decreased of sulphide concentration in PFBR 1, but removal was not observed. On the other hand PFBR 2 showed in this period a maximum removal of about 38 g S $m^{-2}d^{-1}$ of sulphide with effluent sulphide concentration in the range from 0.1 to 0.8 mg S $m^{-2}d^{-1}$.

4.2.2 Sulphur balances calculations

The sum up of the sulphur of all recorded sulphur-species (sulphide, elemental sulphur, sulphite, thiosulphate and sulphate as “total sulphur”) is shown in Figure 20.

Total sulphur resulted in a net sulphur fixation especially in the experimental phase A with 1000-1700 mg S $m^{-2}d^{-1}$ (see Figure 20). Such sulphur deposition was already reported by Winter (1985) in the case of an industrial wastewater loaded with SO_4^{2-} and $S_2O_3^{2-}$ (area-specific load of 1.1 g S $m^{-2}d^{-1}$), showed that constructed wetlands can act as an important sink for sulphur. Two percent of the load was retained in the soil, 31 % as S^o, 25 % as organic S (mainly in humic matter), 15 % as sulphate and 11% as sulphide. Both microbial and abiotic processes are responsible for these transformation processes.

The increase of the hydraulic retention time to 10 d, in experimental phase C showed no removal rate in PFBR 2.

In Figure 21 are shown the total sulphur accumulation of the both reactors during the three experimental phases A, B and C. In the phase C is for PFBR 2 a "redisolution" of also already deposited sulphur obvious.

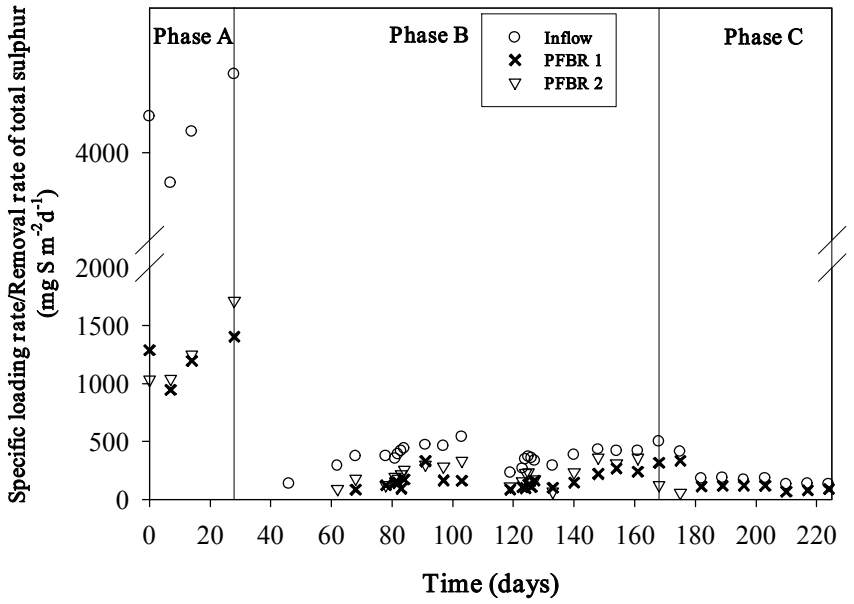

Figure 20 Specific loading and removal rate of total sulphur of the Planted Fixed Bed Reactor.

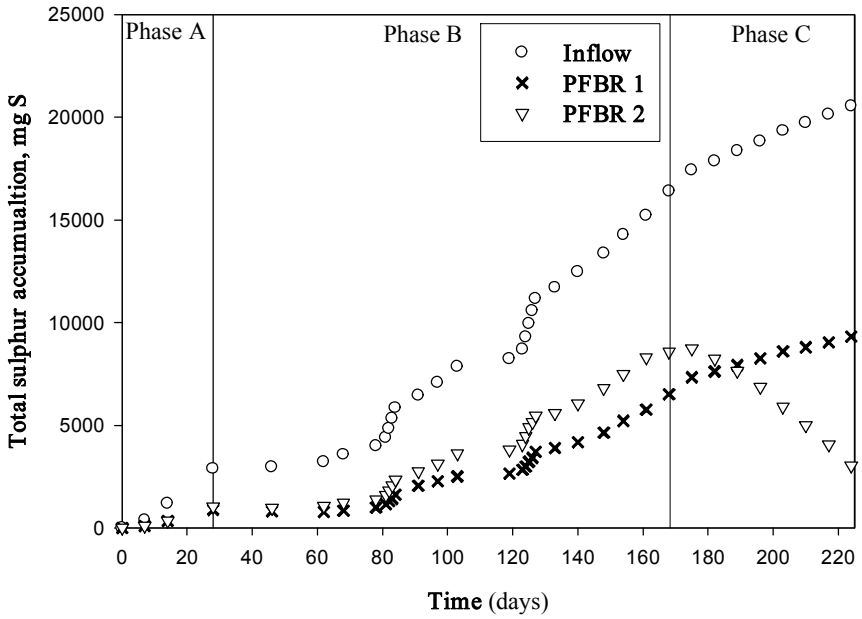

Figure 21.Total sulphur accumulation of the both Planted Fixed Bed Reactors

4.2.3 Nitrogen species /removal

The mean ammonia-nitrogen inflow concentration with 36 ± 3.6 mg N L^{-1} was for all experimental phases approximately the same (Figure 22a).

Under the condition of the experimental phase A (sulphate rich medium), the effluent NH_4^+-N concentration showed differences; whereas in PFBR 1 the ammonium nitrogen decreased, in the PFBR 2 the concentration increased. At the end of this period (phase A) both reactors reached concentration of 25 and 18 mg NH_4^+-N L^{-1}, respectively.

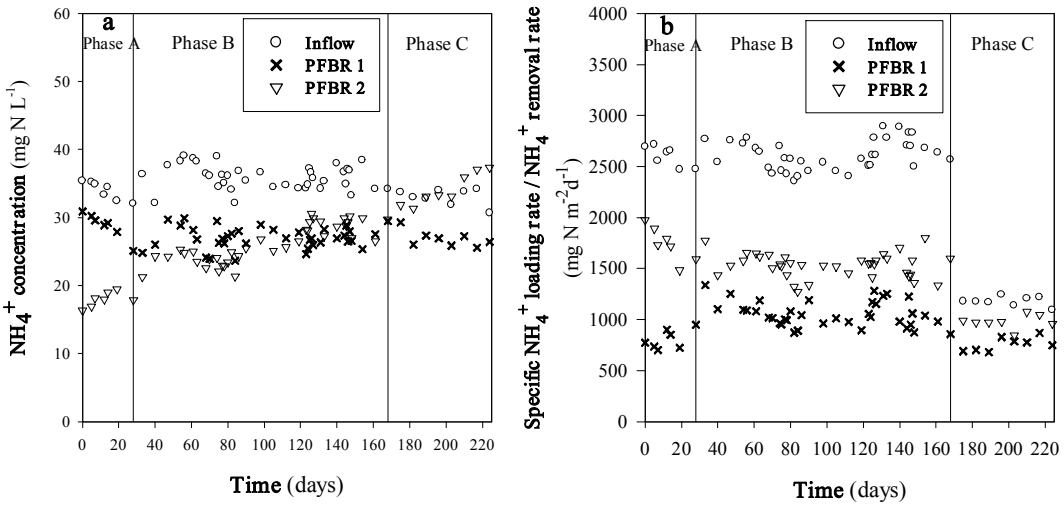

Figure 22 Ammonia concentration a) and specific loading and ammonia removal rate b) of the Planted Fixed Bed Reactor.

During the phase B (low sulphate inflow concentration), the NH_4^+-N concentrations of both reactors showed a similar behaviour, especially at the end of this period ammonium effluent levels reached a similar value of about 30 mg NH_4^+-N L^{-1} (see Figure 22a).

By increasing the HRT to 10 days (phase C) the average effluent concentration for both planted reactor present significant differences in comparison to the phase B. Mean NH_4^+-N values about 28 mg NH_4^+-N L^{-1} were observed for PFBR 1. At the end of the experimental phase C in PFBR 2 the concentration reached levels above the inflow.

Nitrite and nitrate-nitrogen outflow concentration in all PFBR's during all the three experimental phases (A, B and C) was below 0.5 mg L^{-1}. Nevertheless, it is impossible to evaluate their significance as electron acceptor for the oxidation of the organic matter as well as for the sulphide.

The reactors 1 and 2 showed high differences concerning the ammonia removal rates during the experimental phase A (see Figure 22b), whereas the removal in reactor 1 showed an increasing tendency in reactor 2 a decreasing tendency was observed. When the sulphate inflow load was decreased (phase B, see Table 9) the specific ammonia nitrogen removal rate varied with no obvious tendency in a range of 701 – 1,337 mg N $m^{-2}d^{-1}$ in PFBR 1 and 1,271 – 1,974 mg N $m^{-2}d^{-1}$ in PFBR 2.

By decreasing the inflow loading rates under the experimental condition of phase C (increase of the HRT to 10 days, see Table 9) also the specific ammonia nitrogen removal rates decreased with a relative stable mean value of about 760 and 979 mg N m^2d^{-1} for PFBR 1 and PFBR 2 respectively.

Mbuligwe (2004) reported removal rates of ammonia about 2.69 and 2.73 g N $m^{-2}d^{-1}$ for planted beds with *Typha* and *Colocasia*, respectively, treating anaerobic pre-treatment domestic wastewater. In this study the removal rate was in the range from 0.55 to 1.91 g N $m^{-2}d^{-1}$ for PFBR 1 and from 0.85 to 2.26 g N $m^{-2}d^{-1}$ for PFBR 2 with *J. effusus*. It is possible that some differences in performance between this study and that of Mbuligwe (2004) are attributable to different wastewater characteristics and

environmental conditions.

The mean ammonia removal efficiency in the idealized laboratory system in PFBR 2 during the experimental phases B and C reach to 73 %. Wiessner et al., (2005a) reported in the same system using sulphur-limited wastewater, ammonia removal of approximately 82 %. Although there are differences in the synthetic wastewater the ammonia removals are well compared.

Although the outflow concentration of ammonia in the phase C in the PFBR 2 was higher than the inflow (Figure 20a) removal rates was observed (Figure 20b). It could be explained due to the high evapotranspiration observed in this phase (Figure 22d). In this experimental phase the evapotranspiration rate was in the range from 63 to 90 % of the inflow for PFBR 2 (27 to 37 L d^{-1}) and from 50 to 69 % (17 to 24 L d^{-1}) for PFBR 1.

4.2.4 Carbon removal

The inflow concentration of dissolved organic carbon was highly stable during all three experimental phases A, B and C of about 38 mg L^{-1}.

Both PFBR showed outflow concentration within the range of 3 – 11 mg L^{-1} during all three experimental phases (Figure 23). More than 80 % was removed from the inflow.

Wiessner et al., (2005a) have investigated the sulphate reduction and the removal of carbon and ammonia in a laboratory-scale wetland-system, using a wastewater higher in BOD_5 (2.8 times), ammonia (1.4 times) and SO_4^{2-} (2.3 under sulphate rich medium and 105 times under deficient medium). Although there are differences in the synthetic wastewater the result from this study for ammonia and carbon removal are comparable with those by Wiessner et al., (2005a). No clear correlation of S-dynamics with other removal processes could be observed.

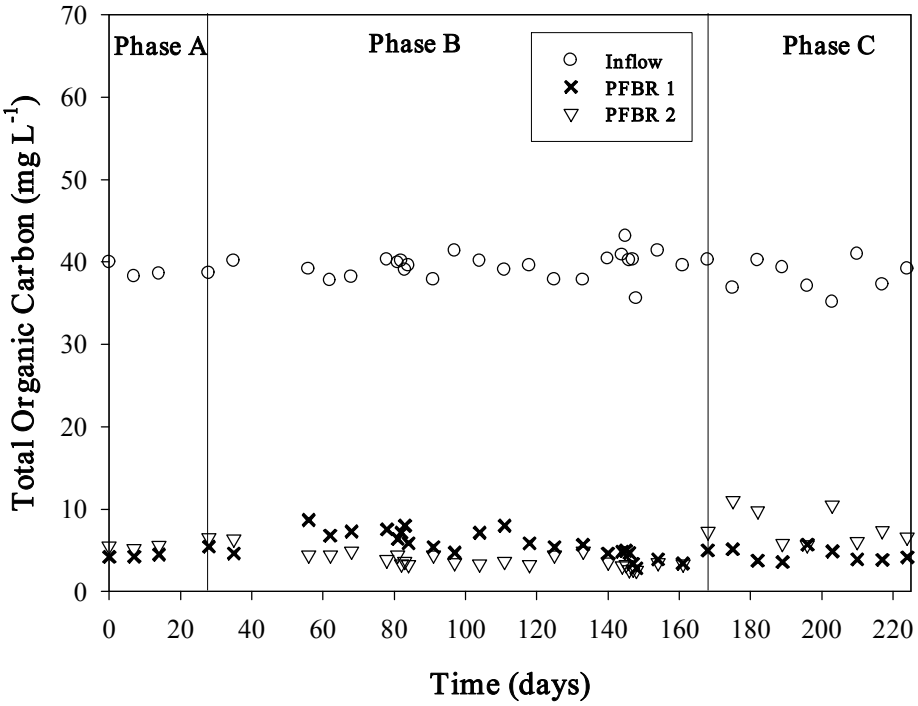

Figure 23 Total organic carbon concentrations of the Planted Fixed Bed Reactors

4.2.5 Further parameters (shoot density, EVT, Eh and pH)

During the experimental phases A, B and C the plant shoot density of both reactors increased steadily (Figure 24a). The initial shoot density in the PFBR 2 was 1.4 times higher than in the PFBR 1 (9,972 and 6,978 shoots m^{-2} respectively). The shoot density increased almost at the same rate. Nevertheless, especially at the beginning of the experiments (phases A and B) big differences concerning the green shoots density were observed. Later on (in the experimental phase C) the difference almost disappeared.

Although total plant density showed similar behaviour; green shoots at the beginning had significant differences (Figure 24b). While green shoots in PFBR 2 in phases A and B was stable, PFBR 1 showed a decrease in phase A. In experimental phase B the plants recovered and later on in phase C showed similar values like in PFBR 2.

Both reactors differed concerning their mean redox potentials within their root-zones (see Figure 24c). In reactor 2 with the higher initial plant density (Figure 24a) was always recorded a higher value (up to 200 mV) than in the reactor 1. Especially at the beginning of the experimental phases (phase A) in the reactor 1 a very low redox potential in the range of -210 to - 270 mV was recorded. It means in the pore water of reactor 1 were conditions which were favourable for microbial dissimilatory sulphate reduction (Boon, 1995; Jackson and Myers, 2002; Choi et al., 2005). Furthermore, within the experimental phase B and C (up to day 120) in the pore water a high oscillation of Eh was observed (see Appendix E). Such an oscillation, but by day time related was reported by Wiessner et al., 2005b using a similar planted fixed bed reactor. It is to assume that under distinct conditions in the pore water within the reactor with permanent mixing macro-gradients on the rhizoplane are permanently "disturbed" by which the conditions within the pore water very fast reflecting the status at the rhizoplane. Under conditions of low redox buffer capacity especially daily variations of oxygen-input by helophytes get visible.

During the experimental phase C (HRT of 10 d) the redox value of both reactors reached a maximum. The PFBR 2 was characterized by a positive value with a maximum of +318 mV indicating oxidizing conditions. In fact in the PFBR 2 the sulphide showed removal efficiency in the range from 67 to 91 %. These values correspond to specific retention of 24 and 38 mg S $m^{-2}d^{-1}$, respectively. In the PFBR 1 only at end of this experimental period C was possible to see som8e sulphide removal, but not significant.

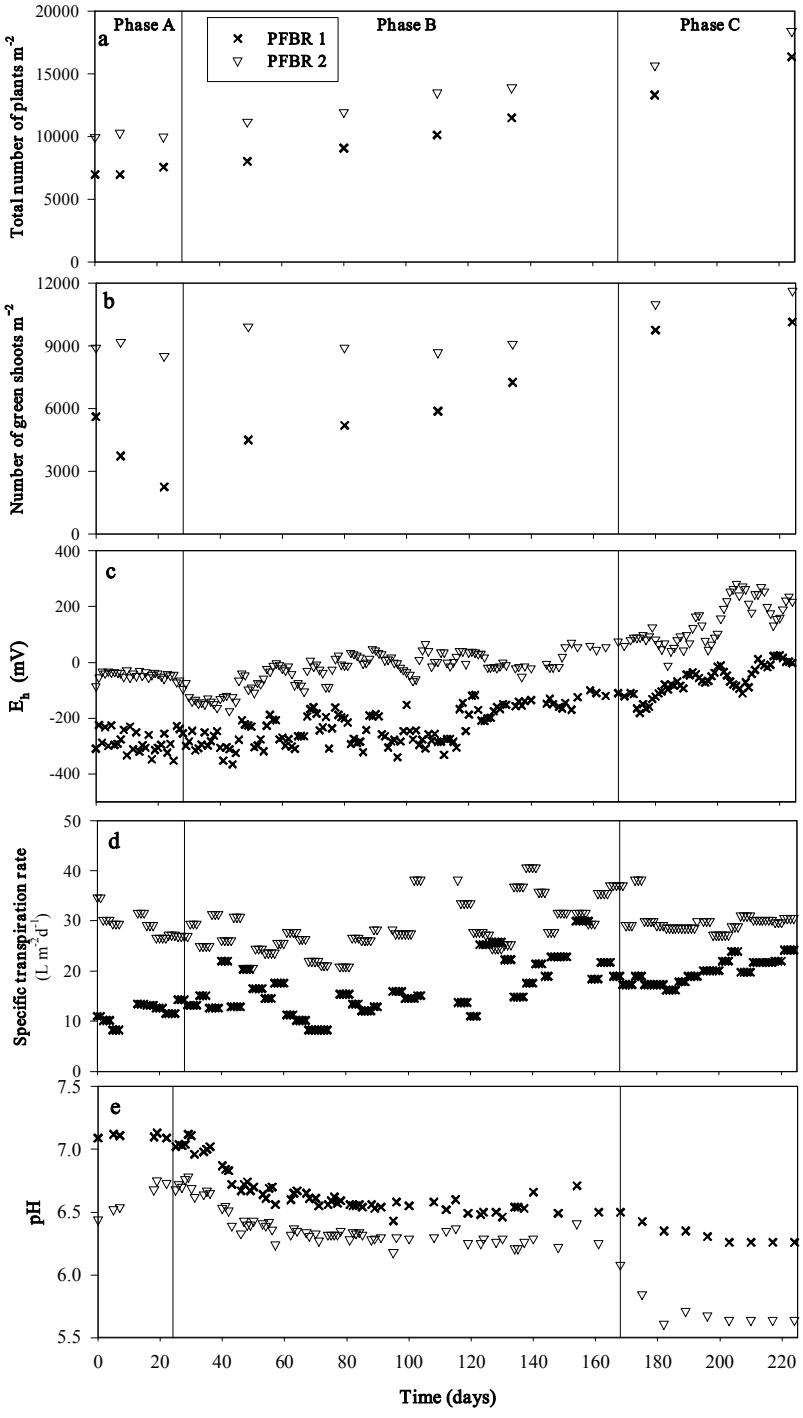

Figure 24 Number of total shoots a), green shoots b), redox potential c), specific transpiration rate d) and pH e) of the both Planted Fixed Bed Reactors.

The higher green shoot density in reactor 2 (Figure 24b) probably resulted to higher mean redox values in comparison to reactor 1 (Figure 24c).

The corresponding specific evapotranspiration (EVT) rates are shown in Figure 24d. The PFBR 2 showed higher and stable evapotranspiration rates in the range of 21 to 40 L $m^{-2}d^{-1}$ in comparison to the PFBR 1 in a range of 8 to 30 L $m^{-2}d^{-1}$.

While the pH in PFBR 1 stayed relatively unchanged in the range of 7.1 – 7.13 (Figure 24e) during experimental phases A the pH of the PFBR 2 rose up from 6.4 to 6.7. During the phase B the pH was decreased slightly reaching low values of 6.5 and 6.2 for PFBR 1 and PFBR 2 respectively. In experimental phase C a clear tendency of decrease pH could be observed especially in PFBR 2 in which the lower values of 5.6 was reached in this final period. The drastically change in the pH values in phase C for PFBR 2 are produced during S^o oxidation to SO_4^{2-} lowered the pH value from 6.1 to 5.6 in a short period of time (See Figure 24e and Figure 19a, b).

4.2.6 Statistical evaluation

The principal component analysis (PCA, Figure 25) summaries the results obtained when comparing inflow and outflow samples for both reactors (PFBR 1 and PFBR 2) in the experimental phases A, B and C. The amount of variation explained by first and second principal components represented 68.4 % of the total variation. Principal component 1 accounted for 40 % of total variability and was mainly loading to ammonia, sulphate, sulphide and pH. Principal component 2 accounted for only 28.4% of total variability and was loading with TOC.

PCA allowed a clear separation of the three experimental phases on the basis of the first two principal components, emphasizing outflow concentration changes of the planted

fixed bed reactors according to the experimental phases. The changes in the inflow (see Table 9) are well differentiated along the first principal component for each of the phase and gathered on the right part of the diagram.

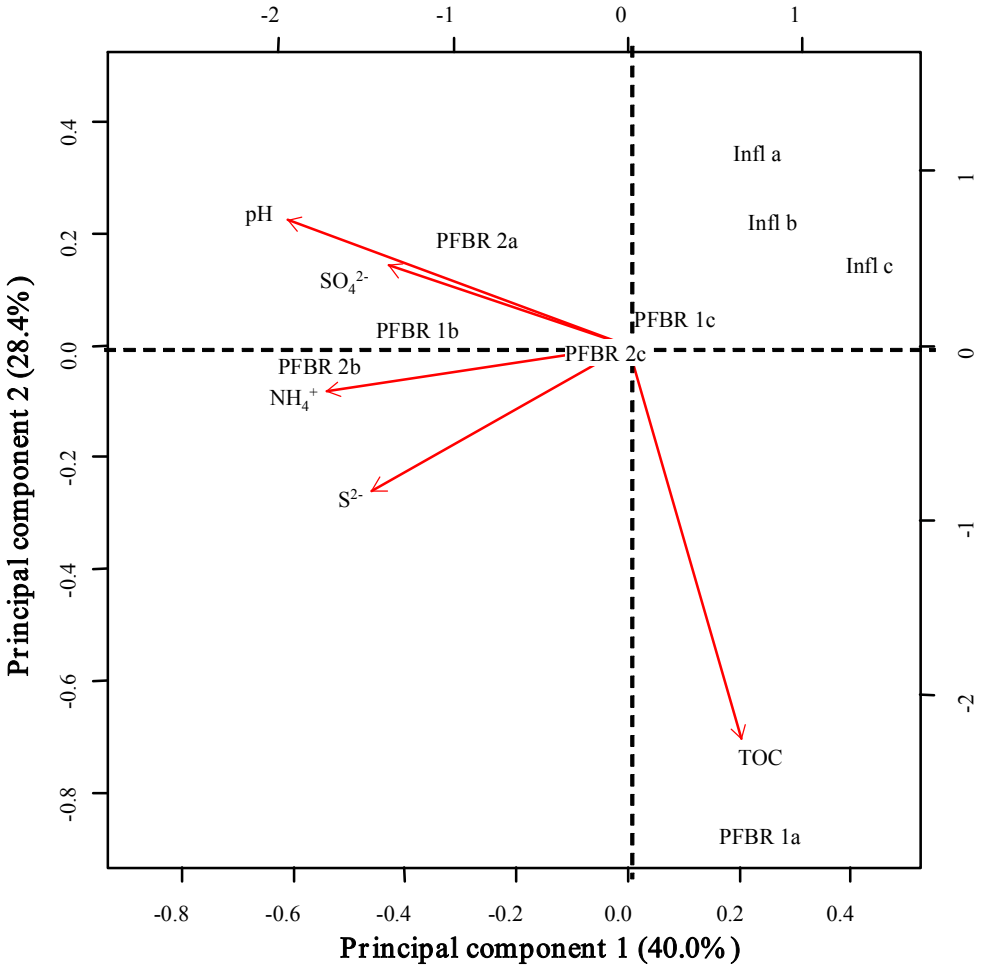

Figure 25 PCA ordination plot of water samples according to sulphur species, ammonia and TOC in the Planted Fixed Bed Reactors during experimental phases A, B and C. A code identifies each bed and phase: the letters refers to inflow (Infl) and Planted Fixed Bed Reactor (PFBR) name, followed by number of reactor 1 and 2 and phase conditions (a, b and c). Ex: Infl a refers to inflow reactors 1 and 2 in phase A; PFBR 1a refers to outflow reactor 1 in condition A.

The outflows of both reactors were similar in experimental phases B and C. The data (PFBR 1b, PFBR 2b, PFBR 1c and PFBR 2c; see Figure 25) clustered together along the principal component 1, suggesting that the outflow of both reactors in those experimental phases were relatively similar. This suggests that parameters related to plant activity and microbial metabolic response were similar for both reactors. In contrast, in phase A PFBR 1a and PFBR 2a were clearly high separated. This changes probably are atributted to the high differents in green plant densities between both planted reactor, as it was found in Figure 24b.

The changes in the phase A in reactor 1 (PFBR 1a) are associated with COT concentration.

Ammonia, sulphate, sulphide, and pH are positive correlative each other along the principal component 1. A negative correlation between pH and TOC was observed along the second principal component.

4.2.7 Specific removal rate of sulphur species in the PFBR

The data of the mean specific sulphur species removal rates (sulphide, thiosulphate, sulphite, sulphate) and the shoot density during the experimental phases A, B and C are shown in Table 13. The removal efficiencies are given brackets.

Table 13 Specific sulphur species removal rates and efficiencies in the Planted Fixed Bed Reactors during the experimental phases A, B and C (experimental condition: see Table 9).

Sulphur species	Specific removal rate (mg S $m^{-2}d^{-1}$)					
	Phase A		**Phase B**		**Phase C**	
	PFBR 1	PFBR 2	PFBR 1	PFBR 2	PFBR 1	PFBR 2
$S_2O_3^{2-}$	90.9 (77%)	111.7 (89%)	163.1 (87%)	183.1 (94%)	102.2 (97%)	103.6 (100%)
SO_4^{2-}	1026.3 (28%)	1223.7 (31%)	-	-	24.4 (41%)	-
SO_3^{2-}	16.5 (54%)	24.4 (75%)	-	-	-	-
S^{2-}	-	-	-	-	3.4 (13%)	20.4 (82%)
Shoots density, shoots m^{-2}	7692	10302	12912	15110	16374	18407

During the experimental phase A PFBR 2 with a shoot density 1.3 times higher than in PFBR 1 showed in general higher specific removal rates and efficiencies. The main sulphur species removed in both reactors were sulphate and to a less extent thiosulphate. In both reactors no net removal of sulphide during this phase was observed. Although the outflow concentration of sulphate in phase A in the PFBR 2 was higher than the inflow (see, Figure 19b) removal rates was observed (see Table 9). It could be explained due to the high evapotranspiration observed (Figure 22d) which reached to up 44% of the inflow (35 L d^{-1}), while only 24% (14 L d^{-1}) in PFBR 1 was observed.

The changes of the experimental conditions with experimental phase B (reduced sulphate concentration, see Table 9) caused a significant effect on the specific removal rates in both reactors. During this experimental phase only a net removal of thiosulphate was observed. The PFBR 2 with a shoot density 1.2 times higher than PFBR 1 showed a slighly higher specific thiosulphate removal rate (183 mg $S_2O_3^{2-}$-S $m^{-2}d^{-1}$) in comparison to PFBR 1 (163 mg $S_2O_3^{2-}$-S $m^{-2}d^{-1}$).

During experimental phase C PFBR 1 showed a removal rate of thiosulphate, sulphate and sulphide while in PFBR 2 only thiosulphate and sulphide was observed. The main sulphur specie removed in both reactors was thiosulphate with similar values above 100 mg $S_2O_3^{2-}$-S $m^{-2}d^{-1}$. During this experimental phase PFBR 2 showed only a slight higher shoot density (1.1 times), than PFBR 1.

The main characteristic of the experimental phase C was that both reactors removed sulphide. In PFBR 1, only 13 % while in PFBR 2, 82 % of sulphide was removed This removal correspond to an area specific removal rate of 3.4 and 20.4 mg S $m^{-2}d^{-1}$, respectively.

4.2.8 Conclusions

In model experiments for subsurface flow constructed wetlands the possibility to treat sulphide containing effluents (like they are generated in case of anaerobic treatment of domestic sewage) under distinct conditions was shown.

Aerobic processes (ammonia and sulphide oxidation) and anaerobic processes (denitrification, sulphate respectively thiosulphate reduction, etc.) occur within the root-zone simultaneously.

The sulphide removal is influenced by various factors like concentration of DOC, nitrogen compounds, sulphide, sulphate and other sulphur species, by loading conditions and as it was shown in these model experiments also by the plantation (density and surely other plant related factors).

The sulphate concentration of the domestic wastewater has to be considered as a factor that may control treatment performance. The presence of sulphate can result in both treatment steps (anaerobic digester and wetland) in sulphide formation.

In case of post-treatment of effluents from anaerobic reactors in subsurface constructed wetlands especially the balance of sulphide, sulphate and residual organic carbon of high bioavailability have to be considered because these systems work simultaneously as an anaerobic and aerobic reactor. The formation of sulphide concentrations toxic to the plants have to be prevented by variation of loading rate for instance.

On the basis of the anaerobic digester (UASB) effluent conditions of the Colombian city Bucaramanga laboratory-scale constructed wetland were operated with a corresponding artificial (synthetic) wastewater.

The apparently differing observations regarding the role of wetland plants with respect to sulphur removal signify the need for more studies on this aspect.

4.3 Treatment of artificial sulphide containing wastewater in subsurface horizontal flow laboratory-scale constructed wetlands

4.3.1 Dynamics of S-species

The data of the sulphide concentrations in the inflow area, middle and outflow area of the model wetlands are shown in Figure 26a. Under the running conditions of phase A there were no remarkable differences concerning the sulphide concentrations in both planted beds (W2 and W3). After the flow through the rooted beds the sulphide in the water was completely removed. Less stable conditions were observed in the unplanted control bed (W1).

The changes of the inflow conditions in phase B (doubling the hydraulic load in all three beds and the increase of the sulphate concentration by four times in W3) resulted in clear changes of the sulphide removal. In the middle of all three beds (after the flow path of 50 cm) the sulphide concentration of the pore water was high; in the final period of phase B (about day 198 – 217) there was no longer a large difference between the sulphide concentration in the inflow area and in the middle of the three beds. While the sulphide concentration of the unplanted control bed in the effluent reached the level of concentration of the inflow area in this final period, all sulphide was still removed in both planted beds. These results correspond to an area specific removal rate in the planted beds up of 70 and 94 mg sulphide S $m^{-2}d^{-1}$ for W2 and W3, respectively (Figure 27a).

The increase of the inflow sulphide concentration in all three beds and the sulphate concentration of bed W1 and W2 in phase C resulted in a decrease of the sulphide removal for all three beds; in the unplanted control bed (W1) almost no removal was observed (see Figure 27a).

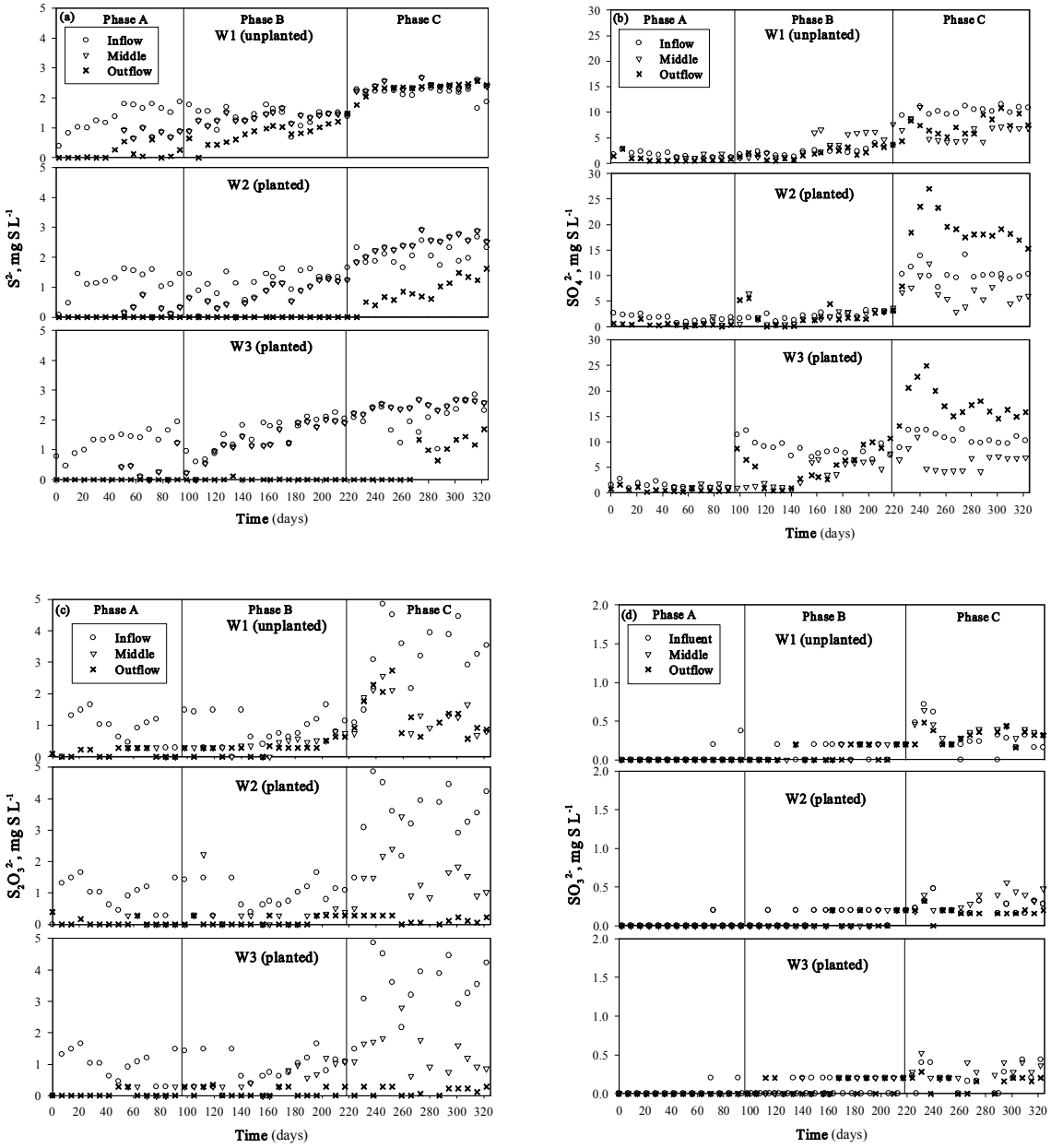

Figure 26 Sulphur concentrations of the inflow, middle and outflow of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

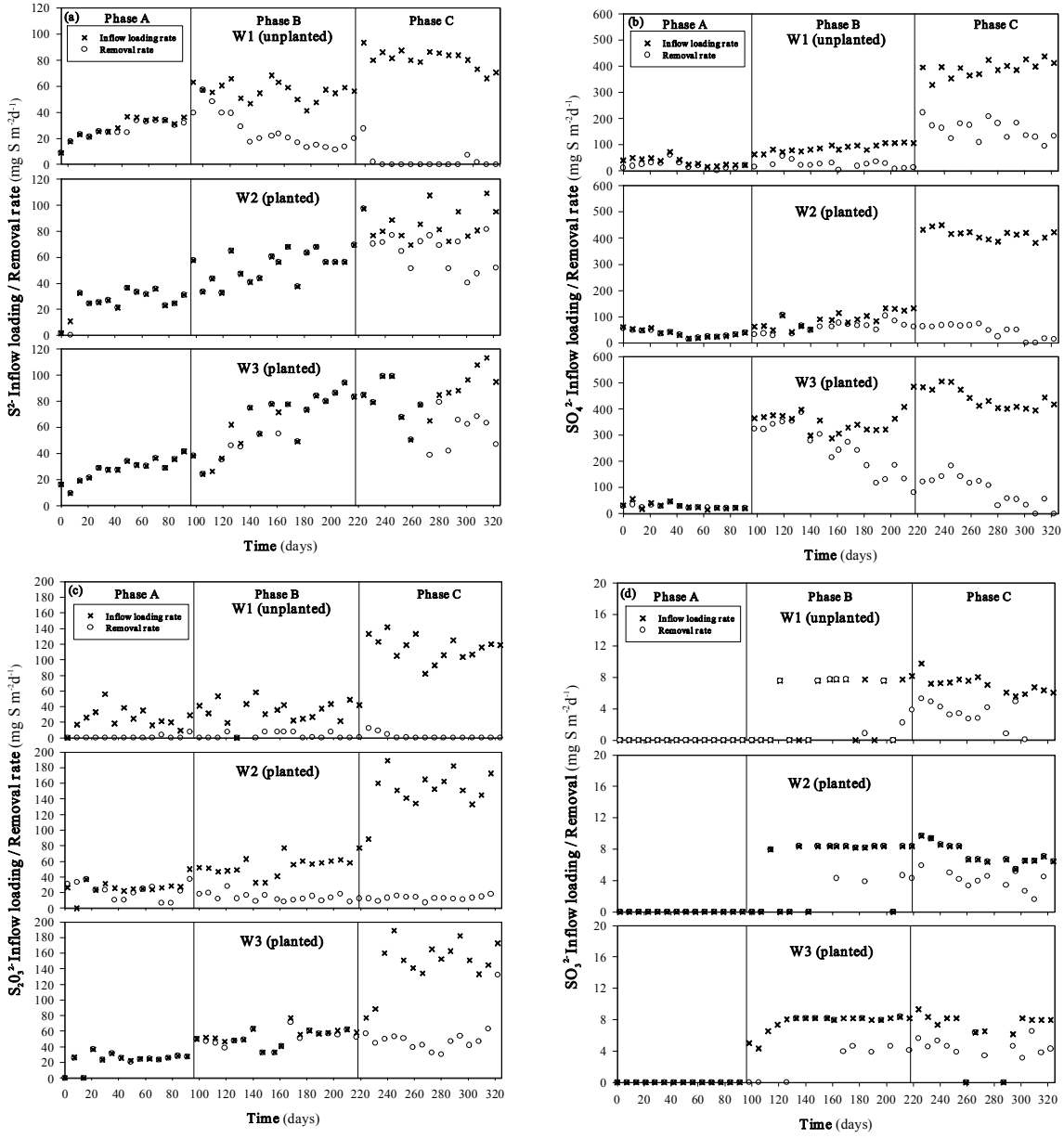

Figure 27 Specific loading rate and removal rate of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

Under the condition of low sulphate inflow concentration (see Table 11) in phase A almost all sulphate-sulphur was removed in the three beds W1, W2 and W3 (see Figure 26b).

In the experimental phase B the increase of the hydraulic loading rate in all three beds (W1, W2 and W3) and the sulphate inflow concentration in the planted bed W3 resulted in beds W1 and W2 showing no changes in comparison to phase A. The increase of the sulphate load in the planted bed 3 (W3) caused instable conditions; in general, the effluent sulphate concentration rose up to a level reaching the inflow concentration at the end of phase B.

By increasing the sulphide concentration in all three beds and increasing the sulphate concentration in bed W1 and W2 the same inflow conditions for all the beds (W1, W2 and W3) were realised in phase C. Therefore the sulphate concentration decreased only slightly below the concentration of the inflow in the control bed (W1). In both planted beds (W2 and W3) the sulphate concentration was only up to half the path of the water flow through the beds (middle) below that of the inflow concentration. During the further flow until the effluent area the concentration increased to a level higher than that of the influent (see Figure 26 b). Because of the water loss due to evapotranspiration in both planted wetlands (83 and 76 % of the inflow for W2 and W3, respectively) sulphate removal rates was observed (see Figure 27b).

Thiosulphate sulphur (probably formed by oxidation of sulphide or other reactions) with an inflow concentration in the range of 0.5 to 1.9 mg S L^{-1} in the experimental phases A and B and 1.5 to 5 mg S L^{-1} for phase C was well transformed with outflow concentrations of all beds of less than 0.3 mg S L^{-1} for phases A and B (see Figure 26c). After the inflow parameter change in phase C (see Table 11) thiosulphate concentration in the effluent for the planted beds stayed nearly constant below 0.3 mg S L^{-1}; while for the unplanted control bed (W1) the effluent concentration rose up to 2.7 mg S L^{-1}.

During the whole experimental period beside sulphide, sulphate and thiosulphate also elemental sulphur and sulphite were recorded. The maximum inflow sulphite concentration reached up to 1 mg S L^{-1} with outflow concentration below 0.48 mg S L^{-1} (Figure 26d). Elemental sulphur (suspended) was found only in the unplanted control bed (W1) during experimental phase C reaching an effluent concentration in a range of 2.3 to 5 mg S L^{-1} (data are not shown).

In the planted beds an influence of oxidative processes resulting from the plant's active oxygen transport into their rhizosphere can be clearly observed (Armstrong et al., 1990; Sorrell and Armstrong, 1994; Wiessner et al., 2002). So besides the better sulphide removal (see Figure 27a) an increase of the sulphate concentration in the planted beds is especially evident in experimental phase C with a high sulphide inflow load (see Figure 26b). The observed removal rates of up to 94 mg sulphide m^{-2} d^{-1} are considerably lower, several grams per m^2 d^{-1}, than values reported for vertical flow wetlands (Giraldo and Zárate, 2001).

Especially during a long period in experimental phase B of the planted bed W3 ("moderate" sulphide and "elevated" sulphate inflow load; see Table 11) the results show a reduction in sulphate concentration (Figure 26(b)), nevertheless also concomitantly the sulphide concentration decreased as well (Figure 26a). These results support the concept of the existence of multigradients within the rhizosphere of treatment wetlands; meaning there are oxic and anaerobic micro-zones within the same system at the same time (Bezbaruah and Zhang, 2004; Wiessner et al., 2005b).

4.3.2 Sulphur balance calculation

In Figure 28 the calculation results of the specific total sulphur (see 3.5.5) loading and removal rates (see 3.5.6) are shown. During experimental phase A no striking

differences of the total sulphur removal rates with a mean value of about 80 mg $m^{-2}d^{-1}$ in all three beds could be observed.

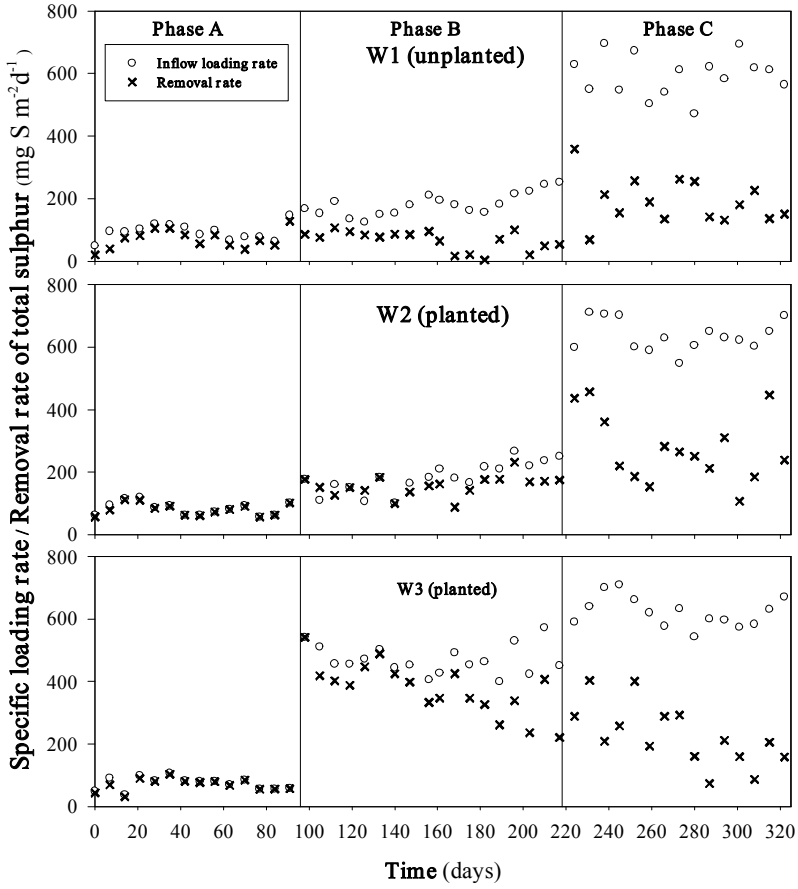

Figure 28 Specific loading rate and removal rate of total sulphur of the subsurface horizontal laboratory-scale constructed wetlands.

Despite almost the same conditions during experimental phase B for bed W1 (unplanted) and W2 (planted) a higher value of the specific removal rate of about 156 mg S $m^{-2}d^{-1}$ could be observed for W2 in comparison to W1 with only about 66 mg S m^{-2} d^{-1} (Figure 28). Bed W3 with an elevated sulphate inflow concentration showed an even higher specific total sulphur removal rate in the range of 221 – 542 mg S $m^{-2}d^{-1}$.

The increase of the sulphide sulphur concentration to about 15 mg L^{-1} in experimental

phase C resulted in unstable conditions and especially in W3 the tendency of decreasing the removal rate could be observed.

In case of a very low load (phase A) and the highest load (phase C) (see Table 11) less differences between planted and unplanted beds concerning total sulphur removal can be observed (Figure 28). It can be assumed that in case of low sulphide load (phase A) the oxygen "supply" by surface diffusion is sufficient for oxidizing processes. Therefore sulphide is probably not totally oxidised but only to elemental sulphur, so that sulphur was removed and deposited from a soluble into an insoluble form in the pore water of the model wetland. Such a "partial" oxidation of sulphide is reported either under phototrophic or under oxygen-limited conditions (Annachhatre and Suktrakoolvait, 2001; Ferrera et al., 2004). This partial oxidation at a low oxygen concentration can be realised by abiotic autoxidation or by bacteria (Annachhatre and Suktrakoolvait, 2001; Eun-Ku et al., 2005).

4.3.3 Sulphur loading and removal rates

The relationship between area specific sulphur removal rates (g $m^{-2}d^{-1}$) and loading rates (g $m^{-2}d^{-1}$) are presented in Figure 29.

In phase A (very low loading rate, Figure 29a) sulphide-sulphur removal increased linearly with inflow loading rates for all reactors. Despite almost the same conditions during experimental phase B for unplanted control bed (W1) and W2 (planted) a higher value of the specific removal rate could be observed for W2 in comparison to W1 which presented unstable condition (Figure 29a). Bed W3 with an elevated sulphate inflow concentration showed the same linear tendency like W2 but with higher inflow loading and removal rate.

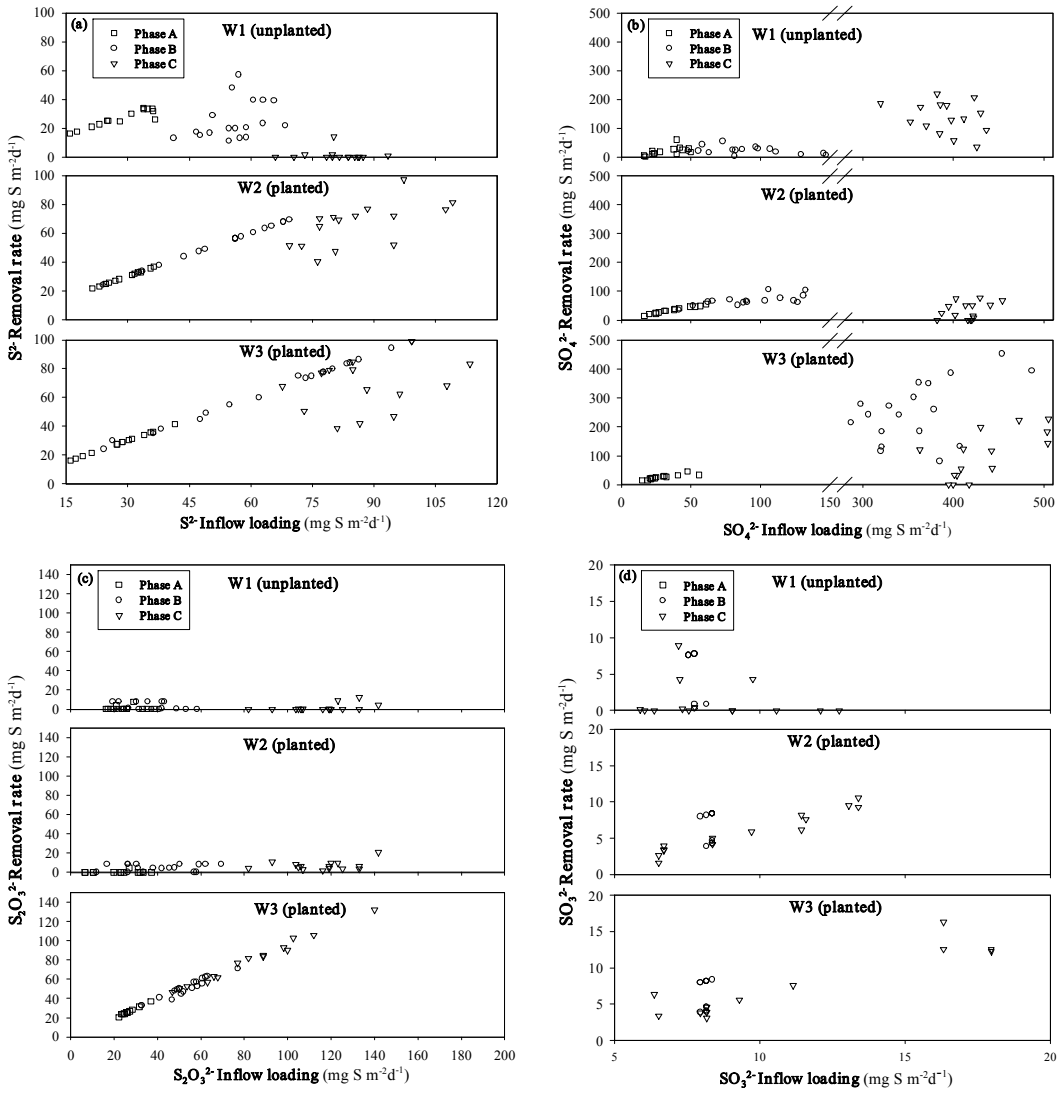

Figure 29 Correlations between inflow loading and removal rate of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

The increase of the sulphide sulphur concentration to about 15 mg L^{-1} in experimental phase C resulted in non sulphide-sulphur removal for unplanted bed (W1) while unstable conditions for both planted beds (W2 and W3) could be observed.

In general relationship between sulphide loading rates and removal rate in phases A and B for both planted beds were obviously linear, with removal rates increasing as loading

rates increase. The relationship at loading rates in phase C appears unstable. These results suggest that specific loading rates higher than 67 mg S $m^{-2}d^{-1}$ for sulphide-sulphur in horizontal flow laboratory-scale constructed wetlands may be cause unstable condition.

It is to mention that in the literature, there are not much information on sulphide removal in horizontal flow wetlands with which the results of this study can be compared.

Sulphate-sulphur removal under the condition of low sulphate inflow concentration (phase A) increased with loading rate in the planted beds W2 and W3 while unplanted bed (W1) sulphate removal was unstable (see Figure 29b). In the experimental phase B planted beds (W2 and W3) showed higher sulphate removal in comparison to the unplanted control bed (W1). Planted bed (W3) with a higher sulphate inflow (see Table 11) showed a higher sulphate removal in a range of 1 to 3 times as W2 (Figure 29b). The increase of the sulphide sulphur concentration in experimental phase C resulted a non stable removal.

With regard sulphate removal rates, Mbuligwe (2004) reported in horizontal subsurface wetlands systems in the treatment of anaerobically pre-treated domestic wastewater (UASB reactor) retention of 0.94 g S $m^{-2}d^{-1}$ for unplanted bed and 1.46 and 1.56 g S $m^{-2}d^{-1}$ for *Typha*, and *Colocasia* units, respectively. In this study the maximum removal rate of sulphate was found for planted bed W3 in phase B 0.45 g S $m^{-2}d^{-1}$, for the unplanted control bed (W1) and planted bed (W2) in phase C with 0.22 and 0.28 g S $m^{-2}d^{-1}$, respectively. Evidently, results from these studies are not well comparable with those reported by Mbuligwe because the sulphate inflow load was higher (5 - 6 g SO_4^{2-} $m^{-2}d^{-1}$) in comparison with this study where the maximum sulphate load was 1.5 g SO_4^{2-}

$m^{-2}d^{-1}$.

Thiosulphate sulphur removal (Figure 29c) was not significant during all phases for unplanted control bed (W1) and planted bed (W2), while planted bed (W3) the relationship at loading rates in all experimental phases showed a linear tendency. This finding suggests that the increase of inflow sulphate concentration from the beginning of phase B in W3 was probably the result of an increase of the bacterial sulphide oxidizing. Furthermore, sulphide outflow appeared in planted bed W3, 40 days later than in W2 where not sufficient inflow sulphate was (phase C, Figure 26a). According with this result thiosulfate is an important intermediate in the sulfur cycle as well as Jorgensen reported in planted and unplanted soil (1995).

Sulphite removal rate in all model wetlands was unstable.

4.3.4 Nitrogen species / removal

The data of the ammonia and nitrate concentrations in the inflow area, middle and outflow area of the model wetlands are shown in Figure 30a and b, respectively.

Under the running conditions of phase A almost all ammonia in both planted beds (W2 and W3) was removed (Figure 30a) while in the unplanted bed (W1) ammonia concentration decreased only about 28 % of the inflow. After the flow through the rooted beds the ammonia (see Figure 30, middle) was reduced to half of the inflow concentration in both planted beds while in unplanted bed (W1) the concentration was closely to the outflow.

In all beds (W1, W2 and W3) during phases B and C non significant differences regarding ammonia concentration in the middle and inflow was observed. Ammonia outflow concentration decreased about 28 % of the inflow concentration in both planted

beds (W2 and W3) (Figure 30a).

There were remarkable differences concerning ammonia removal in the planted beds in comparison to the unplanted control bed (W1). Figure 30a shows that removal took place in the planted wetland reactors as also were reported in laboratory scale reactors by Sousa et al., 2003. The parameter variations of hydraulic retention time, sulphide and sulphate concentration influenced the ammonia outflow concentration.

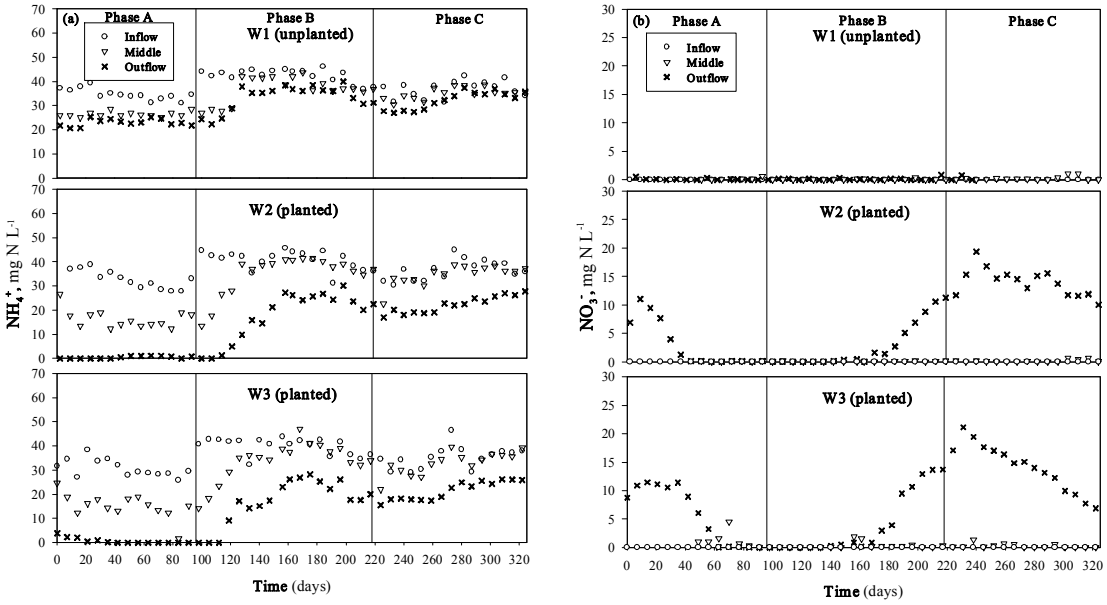

Figure 30 Ammonia a) and nitrate b) concentration of the inflow, middle and outflow in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

Ammonia can be removed from wastewater via a cation exchange, adsorption reaction with organic sediment and soil matrix within a wetland system. Kadlec and Knight (1996) pointed out that removal via this pathway occurs only during the early stage of wetland's life when adsorption sites are still available. In this study gravel was used as soil matrix; by this the ammonia adsorption can be neglected.

Ammonia volatilization within treatment wetlands can provide a removal pathway for nitrogen; however the reaction is pH dependent. The pH values in the experiments (see Figure 33d) were below the pH where substantial ammonia volatilization can occur (Reddy and Patrick, 1984).

Vymazal (2002) found that some subsurface flow wetland beds provide high rates of nitrification, with resultant high quantities of nitrate. The fact that in the experiments nitrate sometimes appears (see Figure 30b) in the effluent at several experimental phases suggests that nitrogen in both planted beds were removed through nitrification and denitrification.

Nitrite concentration in all three beds (W1, W2 and W3) and all the three experimental phases (A, B and C) was below 0.5 mg L^{-1} (data are not shown).

Ammonia removal

For all three beds (W1, W2 and W3) an almost same specific ammonia nitrogen inflow loading rate was realised for the experimental phases A, B and C with about 700 for A, 1,550 for B and about 1,460 mg N $m^{-2}d^{-1}$ for C (see Figure 31).

For the unplanted control bed W1 the specific ammonia nitrogen removal rate varied with no obvious tendency in all three experimental phases A, B and C; the mean value was about 298 mg N $m^{-2}d^{-1}$ (see Figure 31). In contrast to the unplanted control bed W1 both planted beds showed striking elevated removal rates with no significant differences between bed W2 and W3. For these two beds (W2 and W3) the specific ammonia nitrogen removal rate was relatively stable with a mean value of about 681 mg N $m^{-2}d^{-1}$ during experimental phase A. With the increase of the loading rates also the removal rates increased but during the phases B and C with a decreasing tendency. So, at the

beginning of phase B for both beds the specific ammonia nitrogen removal rate amounted to 1,400 mg N $m^{-2}d^{-1}$; later at the end of phase C the value declined to about 800 mg N $m^{-2}d^{-1}$.

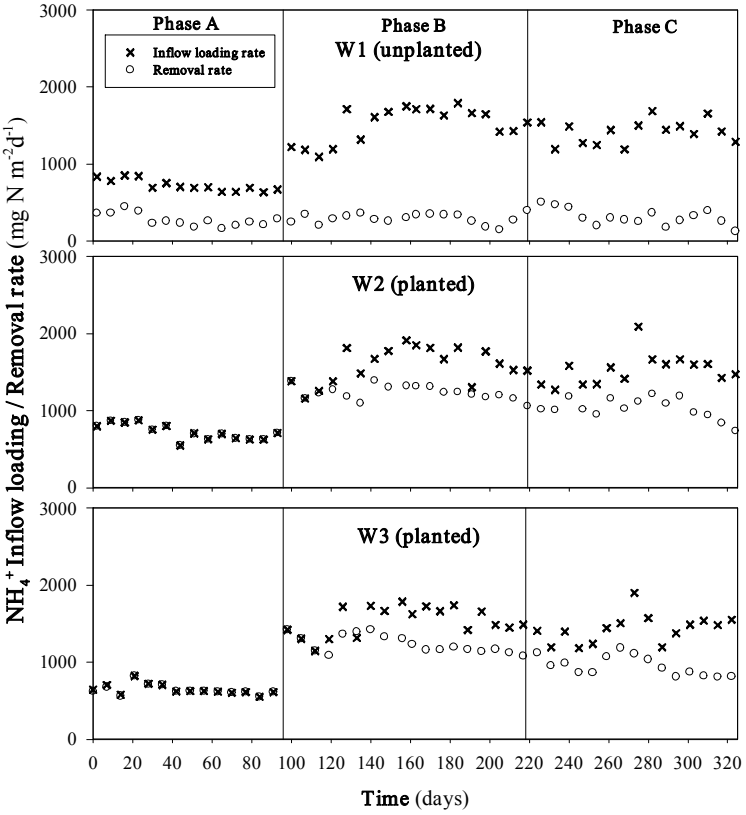

Figure 31 Specific loading rate and removal rate of ammonia of the subsurface horizontal laboratory-scale constructed wetlands.

The plantation showed a clear stimulating effect on the ammonia removal rate. While in the unplanted control bed (W1) an almost constant removal in the range of 150 – 504 mg N $m^{-2}d^{-1}$ was observed parameter variations of hydraulic retention time, sulphide and sulphate concentration influenced the ammonia removal rate within the planted beds in a broader range (600 – 1,400 mg N $m^{-2}d^{-1}$). These ammonia removal rates are in the range reported in literature for subsurface flow constructed wetlands (Sikora et al., 1995; Kuschk et al., 2003). Because of aerobic zones near the plant roots realised by

oxygen transport of the plants to their roots and anaerobic zones more distant from the root surface simultaneous nitrification and denitrification can occur in the "same environment" of the rhizosphere.

Total nitrogen removal

Because of the high evapotranspiration values of the planted beds (see Figure 33b) the occasionally occurrence of nitrate in the effluent has only a small influence on the total nitrogen removal value; by this, area specific ammonia nitrogen removal mainly reflects also the area specific total nitrogen removal.

4.3.5 Carbon removal

The outflow TOC during the experimental phases for the unplanted control bed (W1) varied between 10 – 20 mg L^{-1} and for both planted beds (W2 and W3) the values were somewhat lower at 5 – 10 mg L^{-1}. The area specific removal rate of total carbon in the planted beds were up to 1,504 mg $m^{-2}d^{-1}$ while in the unplanted control bed (W1) the value rose up to 1,012 mg $m^{-2}d^{-1}$ (Figure 32).

The relationship between area specific TOC loading rates and removal rate (Figure 32) in all experimental phases for planted beds were obviously linear, with removal rates increasing as loading rates increase. This result suggests that with higher loading rates more intensive microbial activity in planted beds took place in comparison to the unplanted control bed W1 in which in phase B and C no significant increase of the removal rate was observed.

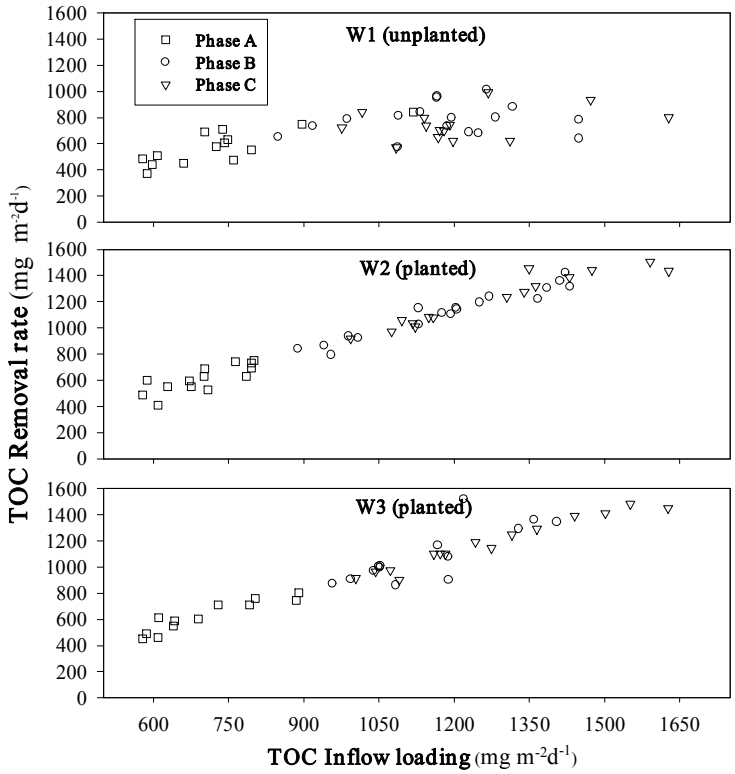

Figure 32 Correlations between the area specific inflow loading and removal rate of total organic carbon-TOC in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

4.3.6 Further parameters (shoot density, EVT, Eh and pH)

During the experimental phases A, B and C the shoot density of the planted beds increased up to 22,397 and 19,333 shoots m^{-2} for W2 and W3, respectively (see Figure 33a). It should be noted that the wetland plants have not got established in the beds and have already produced several new shoots.

Tanner (1996) indicated that *Juncus effusus* showed the highest mean shoot density (4534 shoots m^{-2}) of the eight tested species. The results from this study with *J. effussus* shows a higher density of the plants that reported by Tanner.

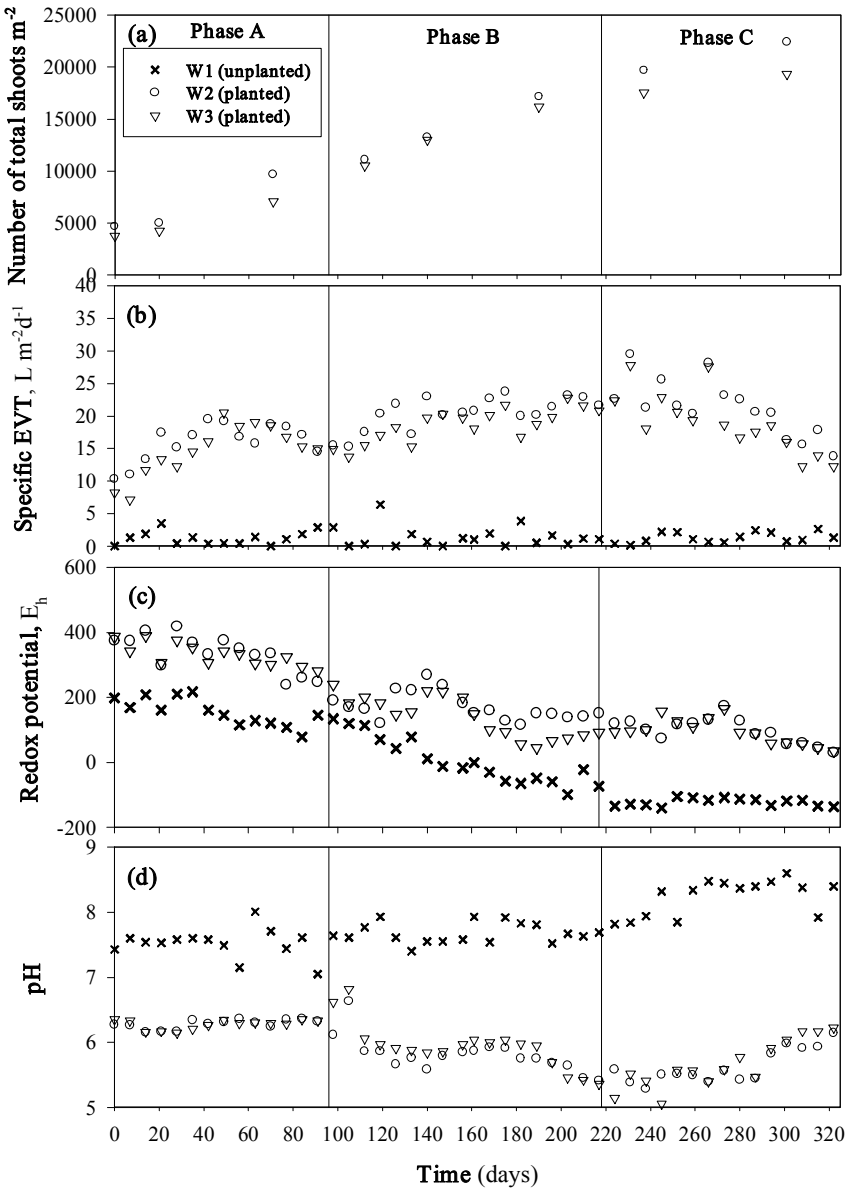

Figure 33 Number of total shoots a), specific evapotranspiration b), redox potential c) and pH d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

A clear decreasing tendency of specific EVT rate in experimental phase C with an elevated sulphide inflow concentration could be observed in Figure 33b. The specific EVT rate in experimental phase C for both planted beds (W2 and W3) decreased by a half with about 30 L $m^{-2}d^{-1}$ at the beginning to about 15 L $m^{-2}d^{-1}$ at the end. Evidently,

results from this study compare well with those reported by Ranieri (2003) with evapotranspiration rates ranging between 21 - 32 L $m^{-2}d^{-1}$ with summer peaks of up to 40 L $m^{-2}d^{-1}$ for a wetland vegetated with *Phragmites* in Italy.

The redox potential (Figure 33c) showed a tendency to decrease during the time for all reactors. A significant difference of about 100 mV between unplanted (W1) and planted wetlands (W2 and W3) was observed. Planted beds showed similar behaviour during the whole experiment with a range of +418 mV at the beginning to about +30 mV at the end.

The positive Eh of the planted beds probably allow better oxidizing condition because the presence of plants, which is indicative of a possibility to transport oxygen into the roof zone. The low sulphide concentration (almost zero) observed in phases A and B (Figure 26a) are according to high oxidizing capacity of the planted beds.

The unplanted reactor showed reduced condition after 160 days of operation, negative Eh and some amount of sulphide concentration in the outflow were observed (see Figure 26a, and Figure 33b).

While the pH in the unplanted bed (W1) stayed relatively unchanged in the range of 7.5 – 8.5 during all experimental phases (A, B and C) the pH of the outflow of both planted beds was significantly lower (5.5 – 6.5) (see Figure 33d). Only in experimental phase C a clear tendency of increase pH could be observed.

The lower pH level in planted wetlands compared to the unplanted shown here is probably caused by ammonium (see Figure 33d) and by sulphide oxidation (Raven and Scrimgeour, 1997).

4.3.7 Statistical evaluation

Figure 34 shows the principal component analysis (PCA) results obtained for all specific inflow loading and removal outflow rates for the unplanted control bed (W1) and the planted beds (W2 and W3) in phases A, B and C.

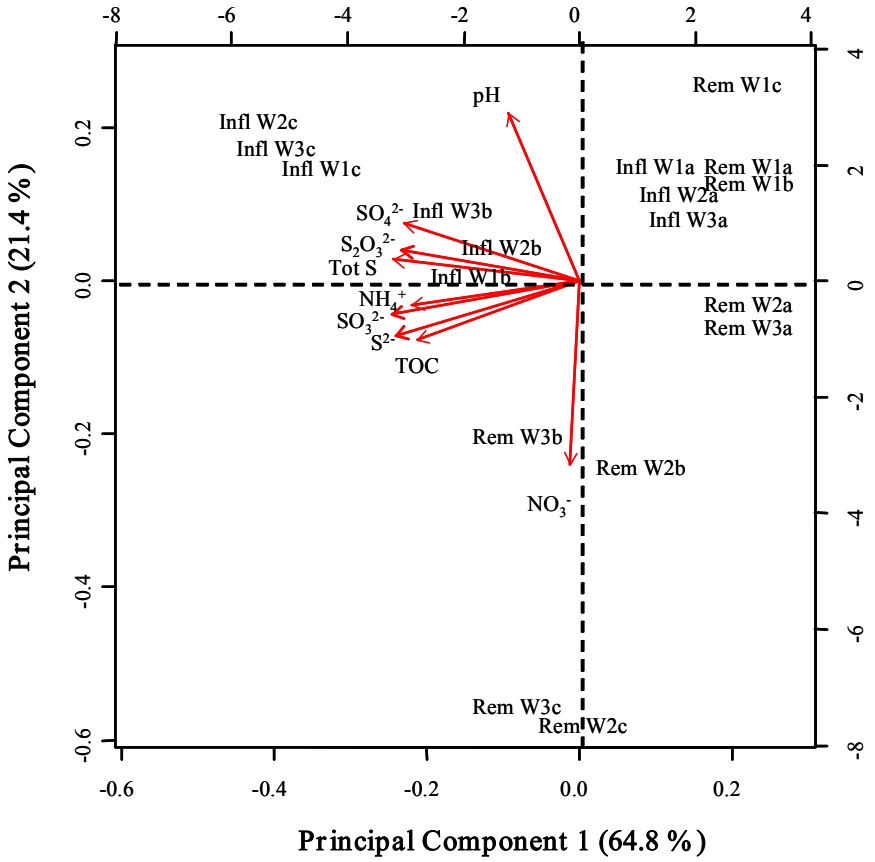

Figure 34 PCA ordination plot of water samples according to sulphur species, ammonia, nitrate and TOC in the unplanted control bed (W1) and planted beds (W2 and W3) during experimental phases A, B and C. A code identifies each bed and phase: the letters refers to inflow (Infl) and removal (Rem) name, followed by number of wetland W1 (unplanted) W2 and W3 (planted) and phase conditions (a, b and c). Ex: Infl W1a refers to inflow wetland 1 phase A.

The amount of variation explained by the first and second principal components represented 86.2% of the total variation. Principal component 1 accounted for 64.8% of the total variation and was mainly loading to ammonia, total sulphur, sulphite, thiosulphate, sulphide and sulphate. Principal component 2 accounted for only 21.4% of total variability and was loading with nitrate and pH.

Samples of the inflow and outflow were mostly related to wetland (planted and unplanted) and experimental phases (a, b and c, see Figure 34). The changes in the inflow (see Table 11) are operating along in the principal component 1 for each phase. A separation of the samples from the phase A to the phase C are operating along the first principal component and they are associated with sulphur species concentration.

PCA allowed a clear separation of the three experimental phases on the basis of the first two principal components, emphasizing concentration changes of the laboratory scale wetland according to the experimental phases. The outflow samples are also well separated by phases along the second principal component. Slight differences were observed when comparing removal rates between both planted beds at the same phases. The samples (Rem W2a, Rem W3a; Rem W2b, Rem W3b and Rem W2c and Rem W3c) clustered together suggesting that the outflow of both planted reactors in those experimental phases display similarities. This suggests that plant activity and microbial metabolic response was very similar between two planted beds.

While the samples of the planted wetlands (Rem W2a, Rem W3a; Rem W2b, Rem W3b; and Rem W2c and Rem W3c) clustered below the principal component 1, the samples of unplanted control bed (Rem W1a, Rem W1b and Rem W1c) was gathered on the right part of the diagram over the prinicpal component 1. This suggests that the physical and biochemical process in planted wetlands (W2 and W3) are differents to

unplanted wetland (W1). Tanner (2002) in New Zealand has also observed that planted wetland beds exhibit overall improved performance compared to unplanted wetlands beds. Based on studies carried out at the University College of Lands and Architectural Studies (UCLAS), Kaseva et al. (2002) have observed as well that planted subsurface flow wetlands perform better that an unplanted one when treating anaerobically pre-treated domestic wastewater.

Microbial wetland plants (W2 and W3) provide suitable sited and conditions for microorganisms which take part in the nitrification processes (see Figure 30b). The nitrate outflow descriptor is negative loading to the principal component 2. More over, Figure 34 showed clearly that nitrification process took place in all experimental phases of the planted wetlands (W2 and W3). The nitrate outflow concentration increased (less removal) from experimental phases A to C, this shift was along the second principal component.

As well as in the Figure 30b and Figure 33d was observed the negative correlation between the pH and nitrate in Figure 34 was also found.

Ammonia, sulphite, sulphide, total organic carbon, total sulphur, thiosulphate, and sulphate showed a positive correlative each other along the first principal component.

4.3.8 Specific removal rate of sulphur species in subsurface horizontal flow laboratory-scale constructed wetlands

The data of the mean specific removal rates of sulphide, thiosulphate, sulphite, sulphate and shoots densities during the phases A, B and C are shown in Table 14. The removal efficiency is given in brackets.

Table 14 Mean area specific removal rates of sulphide, thiosulphate, sulphite, sulphate and shoots densities of the subsurface flow laboratory-scale constructed wetlands during the experimental phases A, B and C (experimental condition: see Table 11).

Sulphur species	Specific removal rate (mg S $m^{-2}d^{-1}$)								
	Phase A			Phase B			Phase C		
	W 1 [a)]	W 2 [b)]	W 3 [c)]	W 1 [a)]	W 2 [b)]	W 3 [c)]	W 1 [a)]	W 2 [b)]	W 3 [c)]
$S_2O_3^{2-}$	0.9 (3%)	20.8 (78%)	26.3 (98%)	2.1 (4%)	12.2 (36%)	50.7 (93%)	1.8 (2%)	10.7 (6%)	46.3 (51%)
SO_4^{2-}	21.1 (65%)	34.8 (95%)	25.4 (88%)	19.3 (22%)	68.2 (76%)	254.7 (71%)	138.1 (32%)	32.1 (8%)	100.8 (23%)
SO_3^{2-}	-	-	-	2.7 (69%)	4.6 (84%)	4.8 (77%)	1.3 (15%)	5.8 (61%)	6.5 (68%)
S^{2-}	26.7 (94%)	28.9 (100%)	28.2 (100%)	25.4 (45%)	52.5 (100%)	63.5 (100%)	1.2 (1%)	66.3 (77%)	69.6 (78%)
Shoots density, shoots m^{-2}	-	10496	8865	-	18255	16184	-	22397	19333

[a)] Unplanted wetland, [b)] planted wetland, [c)] planted wetland

During the experimental phase A the main sulphur species removed in all three wetlands were sulphide and sulphate. From the beginning, the specific thiosulphate removal rates in W3 showed higher values in comparison to the wetlands W1 (unplanted control) and W2 (planted). In this experimental phase the specific sulphide removal rate was similar for all three wetlands with values of 27 - 29 mg S $m^{-2}d^{-1}$ (see Table 14).

Sulphite was no detected in all wetlands systems during this experimental phase. At the end of the experimental phase A, W2 (planted) showed a higher (1.2 times) shoot density in comparison to W3 (planted).

In experimental phase B the unplanted wetland (W1) showed for sulphate and sulphide almost same specific removal rates as in the phase A. The values of the efficiency (%) were lower in comparison to the experimental phase A because of the inflow rate. In both planted wetlands (W2 and W3), the main sulphur species removed were sulphate and sulphide. During this period the shoot density in W2 was 1.1 times higher than in W3. The planted wetland W2 with similar operation condition like the unplanted

wetland W1 (see Table 11) showed two times higher specific removal rates of all sulphur species.

The increase of the sulphide inflow concentration in experimental phase C (see Table 11) affected the specific removal rate in all wetlands. In this period the shoot density in W2 was 1.2 times higher than in W3. Although both planted wetlands (W2 and W3) showed similar specific removal rates of sulphite and sulphide high differences of sulphate and thiosulphate removal was observed (see Table 14).

4.3.9 Conclusions

The results of the experiments substantiate the suitability of post-treatment of anaerobic reactor effluents in subsurface horizontal flow constructed wetlands under the given conditions.

In general higher dynamics of the sulphur transformation with concomitant higher sulphide and thiosulphate removal than in the unplanted control bed could be observed in the planted wetlands.

The results in general show the suitability of constructed wetlands for the removal of reduced respectively partly oxidized sulphur compounds (sulphide, thiosulphate); consequently an increase of the sulphate concentration can be expected.

It was shown that sulphide removal in planted horizontal flow constructed wetlands is limited by the sulphide tolerance of the plants. *Juncus effusus*, for example, is not suitable for the treatment of water with sulphide concentrations of $\geq$ 10 mg L^{-1}. The achieved sulphide removal rates in planted beds were considerably higher than in the unplanted control beds. However, the maximum specific sulphide removal rate of 94 mg sulphide $m^{-2}d^{-1}$ in the planted beds achieved so far is lower compared to the carbon

and ammonium removal rates. It should be noted that sulphide removal was effected by the sulphate concentration in the influent water.

The correlation between the loading and removal rate of sulphide, ammonia and also TOC showed positive correlation during the phase A for planted beds. The findings indicate the significant correlations of sulphide, carbon and nitrogen removal in the rhizosphere of constructed wetlands, particularly under the micro-scale gradient conditions of the root zone environment.

Only for TOC a relatively stable and linear removal rate in a range of 400 to 1,500 mg $m^{-2}d^{-1}$ in the planted beds was observed.

The statistical analysis of PCA indicated that there were significant differences between the unplanted and planted experimental wetlands concerning the removal rates. While less differences between experimental phases in planted beds was observed.

For a detailed understanding, the effects of sulphur transformation on the removal performance in constructed wetlands should be investigated in future experiments, particularly in terms of biotical or abiotical of oxidation of reduced sulphur compounds, competition for oxygen due to oxidation of reduced species, carbon and nitrogen, changes of micro-environmental conditions in the rhizosphere due to redox potentials and sulphur deposits, nutrient mobilization or immobilization, and biofilm formation.