On possibilities of the post-treatment of anaerobic digester effluents with high H_2S loads in constructed wetlands

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1 Introduction

The use of anaerobic sewage treatment in tropical regions is a consolidated practice as primary treatment (van Haandel and Lettinga, 1994; Chernicharo, 1997; van Starkenburg 1997; Foresti, 2001; Wiegant 2001, Jordao and Volschan 2004, Mbuligwe, 2004; Aiyuk, 2004). The attributes of an anaerobic process that render it an attractive and appropriate option include the requirement of low initial investment and energy for operation, lower sludge production and easier maintenance compared to conventional aerobic processes (Van Haandel and Lettinga, 1994; Kalogo and Verstraete, 2001).

However, it is recognized that a sulphate-rich wastewater can cause some significant problems resulting from sulphate reduction in the anaerobic treatment process. In anaerobic conditions the sulphate-reducing bacteria start to work, breaking down the organic matter by sulphate respiration and thus producing hydrogen sulphide. Hydrogen sulphide, H_2S, is toxic to aquatic animal life in very low concentrations and has a very unpleasant odour. The threshold limit value for fresh or salt water fish is 0.5 ppm (EPS, 1984).

Because of the toxicity of sulphide and last but not least also due to the bad odour of H_2S, such anaerobically pre-treated wastewater needs further steps of post-treatment. For sulphide oxidation/detoxification in wastewater several methods are available such as chemical oxidation (Fagan and Walton, 1999; Witherspoon et al., 2004) using hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and iron chloride ($FeCl_3$). Physical methods include the use of activated carbon (Boudou et al., 2003), but the regeneration cycles and the costs of the chemicals are disadvantageous. Another possibility to control the problem is by microbial processes in special bioreactor with phototrophic bacteria but the need of light sources and intensity are not economically appropriate technologies for implementation in Latin America.

Due to restricted financial budgets simple methods/systems like ponds or wetlands are often preferred (Kadlec and Knight, 1996; Al-Malack et al., 1998; Mbuligwe, 2004). The wastewater treatment in constructed wetlands is a relatively new emerging technology with some advantages: no energy for aeration is needed – roots of special plants (helophytes) allow the transport of oxygen to the rooted soil (Armstrong et al., 1990). Different variations of water flow can be realized (surface flow and subsurface flow); by this direct transfer of the volatile H_2S into the atmosphere can be minimized. There are a lot of reports about carbon and nitrogen removal (Kadlec et al., 2000; Vymazal, 2002; Stottmeister et al., 2003; Sousa et al., 2003; Mashauri et al., 2003; García et al., 2004; Kaseva, 2004). Comparably, knowledge regarding the dynamics of sulphur compounds like sulphate, sulphide, elemental sulphur etc., under the gradient redox conditions in constructed wetlands is still limited (Wiessner et al., 2005a).

In the past, little attention has been paid to the sulphur metabolism in constructed wetlands. 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 $m^{-2}d^{-1}$) and without significant concentrations of heavy metals, Winter and Kickuth (1989a) showed that constructed wetlands can act as an important sink for sulphur. However, the importance of the sulphur transformation processes in the case of treatment of domestic wastewater has not yet been sufficiently evaluated (Wiessner et al., 2005a), even more sulphide oxidation, immobilization/remobilization of sulphide and elemental sulphur transformation with other redox processes and the toxic effect of sulphide upon plants and microorganisms.

In model experiments with a laboratory-scale constructed wetland Wiessner et al. 2005a showed that also the sulphate-sulphur of domestic sewage can play an important role as an electron acceptor for the removal of the organic carbon load and influences the ammonia removal.

Knowledge regarding the dynamics of sulphide, elemental sulphur, sulphite, thiosulphate, polythionates, sulphate, etc. and their interactions under gradient redox conditions in planted soil filters is highly limited. According to the interactive potential of sulphur species in wetland systems, it has been ascertained that:

- Sulphur input to wetlands may cause sulphur deposits of elemental and organic sulphur (Winter and Kickuth, 1989b), but the balances were mostly incomplete and there exist no information about the remobilization, particularly under redox-dynamic conditions;

- Sulphate in wetlands initiates Eh and pH changes, C-transformation and, indirectly the mobilization of nutrients (Feng and Hsieh, 1998; Lamers et al., 1998)– all these processes are of importance for carbon, nitrogen, phosphorous-removal but not yet sufficiently evaluated;

- Sulphide may be highly toxic to microorganisms and macrophytes, and is a competitor for the consumption of oxygen (Armstrong et al., 1996a; Chambers et al., 1998; Lee, 1999; Koch et al., 2001; Pedersen et al., 2004);

- The potential microorganisms of the sulphur cycle coexist very closely with all other microorganisms involved in the removal processes in microbial mats and sediment layers, especially under micro-scale gradient conditions (Overmann and van Gemerden, 2000; Liesack et al., 2000; Blaabjerg and Finster, 1998; Wind and Conrad, 1997).

Therefore, the role of sulphide loads of wetlands for the post-treatment of anaerobically treated domestic wastewater should be revisited in terms of treatment effectiveness. The sulphur-cycle processes inside the rhizosphere and their role in the complex network of transformation processes are necessary for better understanding of the “black box” rhizosphere and for optimum design and operation of wetland systems.

Using a system of planted fixed bed reactor- PFBR and planted and unplanted laboratory-scale subsurface horizontal flow constructed wetlands concentrations of reduced sulphur species and hydraulic loading condition were varied and the dynamics of sulphur compounds, removal efficiencies and constitution of the plants were evaluated.

1.1 Objectives

The objective of this work is to assess the effects of the plants on the cycling of sulphur compounds in wetland prototypes. The research was focused on following aspects:

- Toxicity of sulphide to plants

- Nature of sulphur compounds formed within the different zones of the constructed wetland

- The effectiveness of constructed wetlands to remove S-compounds

- Possibilities for improvements of sulphide removal in constructed wetlands

1.2 Problem

The developing countries face the enormous challenges of promoting quality of life usually under very adverse condition of financial resources availability. Sanitation, environmental protection and natural resources conservation are among those items that have deserved less attention and received less investment that needed for the improvement of living conditions. For these countries, the option for low cost systems that provide sanitation associated to environmental protection and natural resources conservation is mandatory.

The applicability of anaerobic reactors for the treatment of domestic sewage is definitively established in tropical and sub-tropical regions. It is well known that effluents from anaerobic reactors cannot be discharged into receiving water without further post-treatment. In this way, the search for post-treatment alternative arose to permit the application of anaerobic reactors even under very restrictive situations.

The search for alternative solutions related to wastewater treatment systems has taken advantages of some favourable environmental condition that amplify the range of applications of non-conventional systems. This is the case of the anaerobic processes for wastewater in some developing countries, including Colombia.

The effluent of Up Flow Anaerobic Sludge Blanket- UASB contains sulphide concentration of about 10-20 mg L^{-1} ("Rio Frio", wastewater treatment plant, Bucaramanga-Columbia, 2004) and the legal limits of sulphide concentration in wastewater are in a range of 1 - 2 mg L^{-1} (Zartner-Nyilas and Deutsch, 2004). It means that anaerobic effluent cannot be discharged directly in the water bodies; a second post-treatment step is necessary. In order to remove these contaminants, biological treatments are preferred technologies rather than physical-chemical methods, which are expensive and may generate toxic residues.

The anaerobic digestion step can be linked with further treatment in a constructed wetland but because of insufficient experiences some questions such as possible sulphide toxicity effects on plants, the transformation processes of the sulphur compounds, the different redox processes turnover in the rhizosphere of helophyte and other influencing factors are unknown.

2 Literature Review

2.1 Chemical methods for H_2S removal in wastewaters

There are numerous chemical compounds available to control H_2S odour. Although these chemicals can oxidize H_2S, their use depends on environmental, economic and operational conditions, which make it highly different for each application.

Chlorine gas (Cl_2), environmental and safety concerns surrounding its use are some of the disadvantages and the use of Cl_2 produces chlorinated by-products (Fagan and Walton, 1999).

Sodium hypochlorite (NaOCl) is an oxidant that oxidizes sulphide to sulphate. This compound does not act selectively for sulphide; therefore, the actual dose required depends on other chemicals/materials present within the wastewater to be treated (Witherspoon et al., 2004). Chlorine is not regenerated in the process, so it may result in a high operational cost. Moreover, in the presence of organic compounds, chlorine oxidation is not attractive due to the formation of undesirable organic chloride compounds.

Hydrogen peroxide (H_2O_2), the oxidation rate of sulphide with hydrogen peroxide is relatively slow (Cadena and Peters, 1998). Twenty to 30 min contact time is normally required for a complete reaction. The mechanism of oxidation of H_2S by hydrogen peroxide is not well understood; however, it is suggested that direct oxidation of sulphide by hydrogen peroxide depends on the reaction with oxygen released during gradual decomposition of hydrogen peroxide. The benefits of its use include dissolved oxygen elevation which helps maintain aerobic conditions and (when used in conjunction with iron) more efficient solids separation through primary clarifiers (Fagan and Walton, 1999).

Ferric chloride ($FeCl_3$), can also oxidize H_2S, complex the sulphides in insoluble ferrous sulphides (FeS or FeS_2) but should be attempted because of the toxicity risk for algal biomass in biological post treatment (Paing et al., 2003).

Ferrous chloride ($FeCl_2$), a precipitating agent that combines with dissolved sulphides, forming an insoluble iron-sulphide complex that precipitates out (Witherspoon et al., 2004).

The Fe^{+2} is not regenerated during the process which means considerable reagent consumption.

Calcium nitrate ($Ca\ (NO_3)_2$), a nitrate-bearing chemical which, when added in sufficient quantities, is capable of utilizing the nitrate for oxidizing sulphide to sulphate (Witherspoon et al., 2004).

Aeration (O_2), this process can be carried out under atmospheric pressure, using oxygen from the air, preferably with the formation of sulphate. Nevertheless the chemical oxidation of H_2S takes place at very low rate in the presence of oxygen dissolved from air. This process is governed mainly by microbial (aerobic) processes existing in wastewater (Bowker et al., 1985). On the contrary the aeration of wastewater enhances the emission of H_2S to the atmosphere because of turbulence action of air (Mamta et al., 1995).

2.2 Biological Methods

Biological treatment method are based on the capacity of microorganisms, including bacteria, yeast and fungi, to transform certain organic and inorganic pollutants into compounds that have very low impact on health and environment.

Biological methods are usually inexpensive compared with most of the physical-chemical treatment methods and also are ecologically cleaner. The most important advantage of biological treatment methods over physical and chemical technologies is the fact that biological processes can be operated at local temperature and pressure (Noyola et al., 2006).

2.2.1 Biological sulphur cycle

Sulphur occurs in surface water in two forms: as (SO_4^{2-}) in aerobic and as hydrogen sulphide (H_2S) in anaerobic waters. Both forms of sulphur are present in wetlands because of the range of oxidation states found in these systems. Natural surface waters receive sulphur from rainfall about 1 to 2 mg L^{-1} as sulphate (Hutchinson, 1975) and from weathering of sedimentary rocks such as dolomite and pyrite. Because many sulphur-containing compounds have low solubility, the sulphate concentration of natural surface waters in open basin is generally low. Hutchinson (1975) cites a mean river sulphate concentration of 16 mg L^{-1}, and Goldman and Horne (1983) list surface water values between 0.2 and 36 mg L^{-1} in lakes and rivers. Natural wetlands typically have sulphate concentration in this same range. Industrialization has increased the concentration of sulphur dioxide (SO_2) in the atmosphere, which can convert to sulphuric acid (H_2SO_4), increasing rainfall sulphur concentration and acidifying surface water.

The sulphur cycle in wetlands, shown in Figure 1 is characterized as an interconnected series of oxidation-reduction reaction and biological cycling mechanisms. Sulphate is an essential nutrient because its reduced, sulfhydryl (-SH) form is used in the formation of amino acids. Because there is usually enough sulphate in surface water to meet the sulphur requirements, sulphate rarely limits overall productivity in wetland systems.

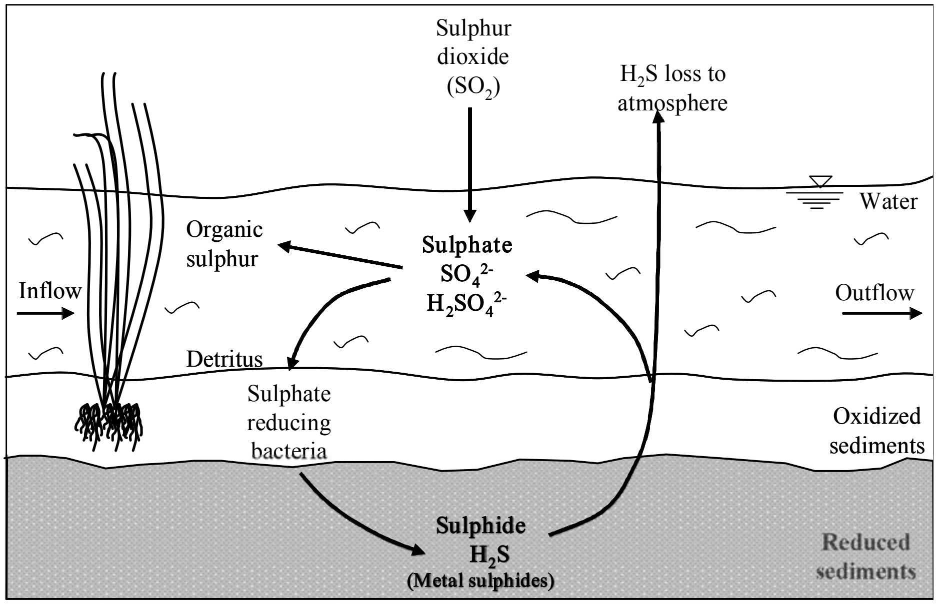

Figure 1 Typical wetland sulphur cycle.

Aerobic organisms excrete sulphur as sulphate. However, upon death and sedimentation, heterotrophic bacteria release the sulphur in detritus in the reduce state, which can result in the accumulation of high levels of hydrogen sulphide in wetland sediments. A second process that transforms sulphate and other oxidized sulphur forms (sulphite, thiosulfate, and elemental sulphur) to hydrogen sulphide in anaerobic sediments is sulphate reduction, mediated by anaerobic, heterotrophic bacteria such as *Desulfovibrio desulphuricans*, which use sulphate as a hydrogen acceptor. Since ferrous sulphide (FeS) is highly insoluble, hydrogen sulphide does not tend to accumulate until the reduced iron is removed from solution. When iron concentrations are low or when sulphate and organic matter concentration are high, significant hydrogen sulphide concentration can occur. Several other metal sulphides are also very insoluble, including ZnS, CdS, and other. Hydrogen sulphide is a reactive and toxic gas with problematic side effects including a rotten egg odour, corrosion, and acute toxicity.

When it is exposed to air or oxygen water, hydrogen sulphide may be spontaneously oxidized back to sulphate or may be used sequentially as an energy source by sulphur bacteria such a *Beggiatoa* (oxidation of hydrogen sulphide to elemental sulphur) and *Thiobacillus* (oxidation of elemental sulphur to sulphate). Photosynthetic bacteria such as purple sulphur bacteria use hydrogen sulphide as an oxygen acceptor in the reduction of carbon dioxide, resulting in partial or complete oxidation back to sulphate.

Wetlands can function as sulphur sink through their internal production and release of hydrogen sulphide as a gas, release of elemental sulphur or methyl sulphide gas, precipitation of elemental sulphur, and precipitation and burial of insoluble metallic sulphides. Adams et al. (1981) measured hydrogen sulphide release from a South Carolina salt marsh as 0.0108 kg $ha^{-1}d^{-1}$. Winter and Kickuth (1989a, 1989b) reported that a root-zone, soil-based treatment system receiving textile wastewaters from a facility in Bielefeld, Germany removed from 80 to 85 percent of the sulphur mass at a hydraulic loading rate of 1.14 cm d^{-1} for a removal rate of 9.6 kg $ha^{-1}d^{-1}$. These authors reported that the majority of this sulphur was largely stored in the wetland soil as elemental sulphur (31 %) and organic sulphur (25 %) and that only a small fraction was released by volatilization to the atmosphere or taken up by plants (1 %).

Sulphate inputs to subsurface flow wetlands are frequently lower when input rates are low and are derived primarily from rainfall and runoff (Bayley et al., 1986). Bayley et al. (1986) measured an annual average net retention of 0.017 kg $ha^{-1}d^{-1}$ for an average removal efficiency of 51 % in a natural black spruce (*Picea mariana*) and sphagnum fen in Ontario, Canada. The portion of this sulphate stored in the organic form was quickly released on a seasonal basis during dry summer condition. Since sulphate inputs in surface wetland treatment systems frequently exceed the biological requirements of wetland biota, wetlands generally are not effective for removal of sulphur (Wieder, 1989).

2.2.2 Sulphate reduction

All plants, animals, and bacteria metabolize sulphur in order to synthesize amino acids such as cysteine and methionine. The sulphur may be assimilated as sulphate or as organic molecules containing sulphur. The reduction of sulphate in biosynthesis is termed *assimilatory sulphate reduction* and can take place in anaerobic or aerobic environments (Goldhaber and Kaplan, 1974; Rheinheimer, 1981; Cullimore, 1991).

Inorganic sulphur species more oxidized than sulphide (sulphate, sulphite, and thiosulfate, for example) can act as electron acceptor in the oxidation of organic matter by bacteria. In the process the sulphur is reduced to sulphide. The reaction is described as *dissimilatory reduction*. The bacteria involved are very versatile.

The sulphate-reducing bacteria like *Desulfovibrio desulfuricans* prefer a pH between 6 and 8, but can function between pH 4.2 and 9.9 (Walhauser and Puchelt, 1966; Baas Becking et al., 1960; Karamenko, 1969; Zehnder, 1988). Sulphate-reducing bacteria can operate at temperatures as low as 0 ^{o}C, and as high as 110 ^{o}C in deep-sea hydrothermal vent sediments. At temperatures higher than 100 to 120 ^{o}C sulphate reduction also proceeds at a measurable rate without bacterial participation (Jorgensen et al., 1992).

2.2.3 Oxidation of reduced sulphur species

Bacteria of the family *Thiobacteriaceae* are probably the most important bacteria involved in sulphur oxidation. Of these, bacteria of the genus *Thiobacillus* have been most studied (Goldhaber and Kaplan, 1974; Cullimore, 1991). The first product of sulphide oxidation abiotically or by *Thiobacillus* is thought to be elemental sulphur according to:

$$H_2S + \tfrac{1}{2} O_2 \rightarrow S^o + H_2O \qquad (2.1)$$

Incomplete oxidation of H_2S at total concentration exceeding the solubility of sulphur ($\approx 5 \times 10^{-6}$ mol kg^{-1}) may lead to the precipitation of colloidal-sized elemental sulphur, which can then react with HS^- to form polysulphides (Boulègue and Michard, 1979; Morse et al., 1987). The successive reactions are:

$$HS^- + \tfrac{1}{2} O_2 \rightarrow S^o + OH^- \qquad (2.2)$$

$$HS^- + (n-1)\, S^o \rightarrow S_n^{2-} + H^+ \quad \text{, with n= 1 to 5 for the polysulphides} \qquad (2.3)$$

Further oxidation of the S^o can produce sulphite. The sulphite may, in turn, be reduced to thiosulfate by reaction with S^o,

$$S^o + O_2 + H_2O \rightarrow SO_3^{2-} + 2H^+ \qquad (2.4)$$

$$SO_3^{2-} + S^o \rightarrow S_2O_3^{2-} \qquad (2.5)$$

or be oxidized to sulphate,

$$SO_3^{2-} + \tfrac{1}{2} O_2 \rightarrow SO_4^{2-} \qquad (2.6)$$

Oxidation of thiosulphate also produces small amounts of trithionate ($S_3O_6^{2-}$), tetrathionate ($S_4O_6^{2-}$), and pentathionate ($S_5O_6^{2-}$) (Goldhaber and Kaplan, 1974).

More recently, Jorgensen (1990) used radioactive ^{35}S to unravel the complex pathway of sulphide oxidation in sediments. He showed that thiosulphate disproportionation to sulphate and sulphide species was a key reaction in anoxic sediments according to:

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \tag{2.7}$$

In Figure 2 are summarized possible oxidation and disproportionation pathways of reduced sulphur species leading toward sulphate that may be mediated by *Thiobacillus*.

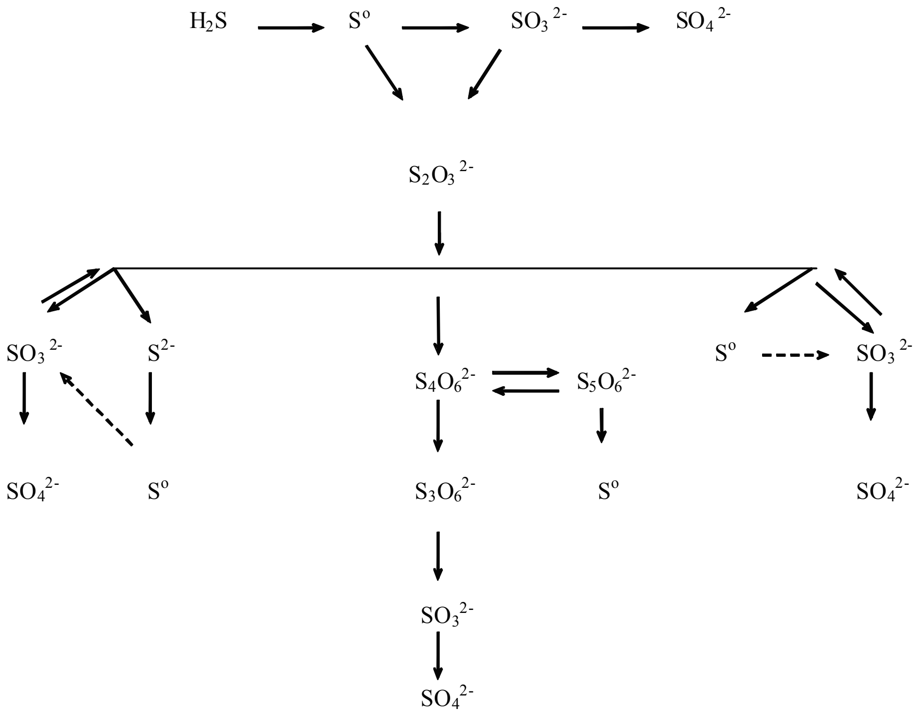

Figure 2 Possible oxidation pathways for reduced sulphur species to sulphate by *Thiobacillus* (Goldhaber and Kaplan, 1974).

2.2.4 Microorganisms of particular interest for Sulphur removal

Among the H_2S oxidizing microorganisms, *Thiobacillus* seems to be particularly suited for engineering applications due to its simple nutritious requirements, its high effectiveness and resistance to toxic substances and the wide pH interval it can tolerate

(Cadenhead and Sublette, 1990).

The most common reaction is a direct oxidation of sulphide to sulphur and sulphate by means of oxygen provided by air. In other cases (*Thiobacillus denitrificans*) nitrate reduction to N_2 allows the oxidation of sulphide to sulphate. Particularly, *Thiobacillus ferrooxidans* raises a very simple and effective process for H_2S treatment in which the oxidant is regenerated by the microorganisms.

Some relevant microorganisms are the following:

Chlorobium limicola – thiosulfatophilum

An autotrophic anaerobic microorganism that uses light as energy which may be a disadvantage due to the associated costs (Cork and Ma, 1982). The system does not depend on oxygen, as oxidation of H_2S takes place in an anaerobic medium in the presence of CO_2. The system favours growth of *Chlorobium* due to the high concentration of H_2S in the reactor, which works like a bactericidal compound inhibiting the growth of other anaerobic bacteria that could compete, such as methanogens. The main advantage of this process is the useful reaction products that are obtained from H_2S and CO_2 according with the following equation:

$$2\,H_2S + CO_2 + h\nu \rightarrow 2\,S^o + CH_2O + H_2O \qquad (2.8)$$

Thiobacillus denitrificans

This chemoautotrophic facultative microorganism with simple nutritional requirement can grow in a heterotrophic environment. The use of these microorganisms has two disadvantages: the slow growth and the sulphate production. (Sublette and Sylvester, 1987; Ongcharit et al., 1990).

Thiobacillus thioparus, T. versutus, T. neopolitanus and T. thioxidans

These microorganisms have been used in pilot plants offering similar characteristic in their behaviour. They do not have a clear advantage over *Thiobacillus denitrificans*, as their growth rates are lower, but they have a lower requirement of ammonium (Cadenhead and Sublette, 1990).

Thiobacillus ferrooxidans

The oxidation of the H_2S to S^o is carried out with ferric sulphate according to the reaction:

$$H_2S + Fe_2(SO_4)_3 \rightarrow S^o + 2\,FeSO_4 + H_2SO_4 \qquad (2.9)$$

Ferric sulphate can be regenerated from ferrous sulphate using *Thiobacillus ferrooxidans* as follows:

$$2\,FeSO_4 + H_2SO_4 + 0.5\,O_2 \rightarrow Fe_2(SO_4)_3 + H_2O \qquad (2.10)$$

The first reaction is highly quantitative avoiding the discharge of H_2S. The oxidation reagent is regenerated, so operational costs are reduced.

This arrangement avoids the problem associated with other H_2S oxidation microbiological processes as H_2S does not have an inhibiting effect on *Thiobacillus ferrooxidans* and SO_4^{2-} is not accumulated in the medium (Asai et al., 1990).

Acidithiobacillus ferrooxidans

The bacterium A. *ferrooxidans* is able to oxidize some sulphur compounds such as thiosulphate ions under aerobic conditions. It is well known (Kocheva and Nonova, 1990) that thiosulphate is unstable in strongly acid medium and decomposes by liberating sulphur, according to the reaction (Zagorchev, 1967):

$$S_2O_3^{2-} + 2\,H^+ \rightarrow H_2S_2O_3 \rightarrow SO_2 + H_2O + S \qquad (2.11)$$

The thiosulphate is stable in alkaline or neutral medium.

Desulfovibrio

Desulfovibrio are able to reduce sulphur compounds, such as sulphate, sulphite or thiosulphate as terminal electron acceptors. Although several studies have been performed with the aim of characterizing the dissimilatory sulphate reduction pathway, it remains poorly understood. The sulphate reduction involves three main steps: (i) sulphate activation to APS; (ii) APS reduction to sulphite and (iii) sulphite reduction to sulphide (Akagi, 1995). The last step is still a matter of controversy with two mechanisms proposed. The first one, described by Chambers and Trudinger (1975) involves the reduction of sulphite to sulphide occurring in a single step through the transfer of six electrons. The second one proposes the formation of trithionate ($S_3O_6^{2-}$) and thiosulphate ($S_2O_3^{2-}$) as intermediates in the sulphite reduction.

2.2.5 Ponds

Ponds are used for the primary and secondary treatment of urban wastewater. Waste stabilization is achieved through solid sedimentation to the pond bottom and anaerobic decomposition of organic matter to carbon dioxide, methane, and other gaseous end products (Metcalf and Eddy, 2005). However, their use is often limited because of the problem of odour release, primarily due to the emission of H_2S.

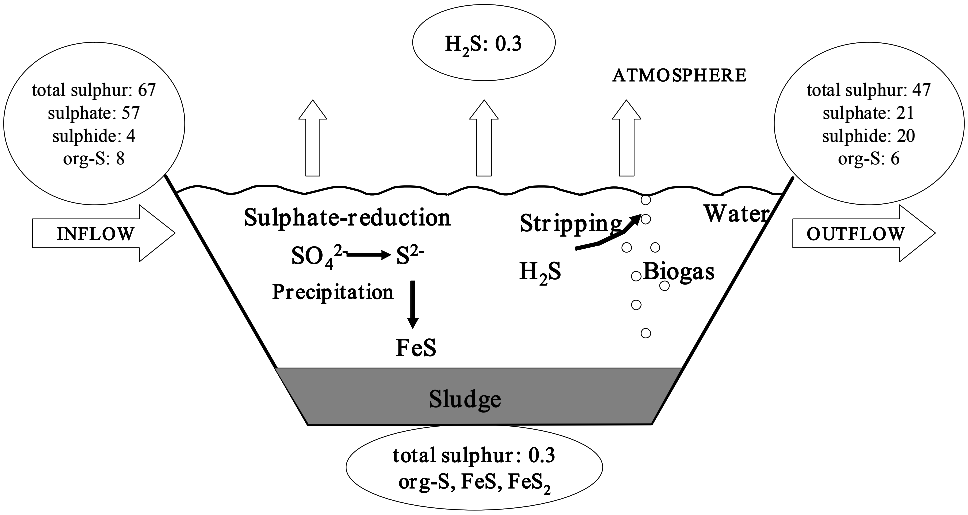

Figure 3 Annual mass balance of sulphur in an anaerobic pond; load expressed in kg S d^{-1} (Paing et al., 2003)

Sulphide formation often occurs in anaerobic and facultative ponds due to the reduction of sulphate under anaerobic conditions, but also to the anaerobic degradation of organic sulphur and the presence of sulphides in the raw wastewater. Since anaerobic ponds effluents are usually treated further in a facultative pond, some facultative bacteria can oxidize H_2S in the presence of dissolved oxygen. The biological oxidation is normally carried out by the photosynthetic sulphur bacteria which requires both light and CO_2 (as the hydrogen acceptor), and H_2S is finally converted into sulphate, the odourless compound (Polprasert and Chatsanguthai, 1989).

According to Paing et al., 2003, the mass balance of sulphur in anaerobic ponds, using a predictive model for the estimation of H_2S emission rate from anaerobic ponds was between 20 and 576 mg S $m^{-2}d^{-1}$, leading to a concentration between 0.2 and 5.2 ppm of H_2S in the surrounded atmosphere and involved a risk of odour nuisances for neighbouring inhabitants. A complete mass balance of sulphur is shown in the Figure 3 (waste stabilization pond system of Méze, France).

The loss of H_2S in the atmosphere and the accumulation in sludge were, thus, very low compared to the flow arriving with the influent. It should be noted that this mass balance was not equilibrated with 67 kg S d^{-1} entering the anaerobic pond and 47.6 kg S d^{-1} "outgoing". This could be explained by the underestimation of the H_2S emission rate or by errors in the estimation of sulphur species in wastewater.

A solution to reduce emission of odorous compounds includes an impermeable cover for gas collection and treatment. It is the more radical solution and its installation and maintenance is relatively expensive. Another solution to reduce emission of odour compounds in ponds includes addition of $FeCl_3$ but there is a risk of toxicity for algal biomass in secondary ponds. Surface aeration and recirculation both increase the operation cost because they need additional energy (Paing et al., 2003).

The choice of a technical solution for the purpose of odour control depends on local conditions and economical considerations (Polprasert and Chatsanguthai, 1989).

2.2.6 Constructed Wetlands

2.2.6.1 Technological aspects

Constructed wetlands can be divided into various types depending on different flow characteristics (Kadlec, 1987; Wissing, 1995). Aquaculture, hydrobotanical and soil systems are considered the main groups. The schemes of some systems are shown in Figure 4.

The basic types of soil-based constructed wetlands are:

- Horizontal surface flow system (with the wastewater level above the soil surface);

- Horizontal subsurface flow systems (with the wastewater level below the soil surface);

- Vertical flow systems (with upstream or downstream characteristics and continuous or intermittent loading.

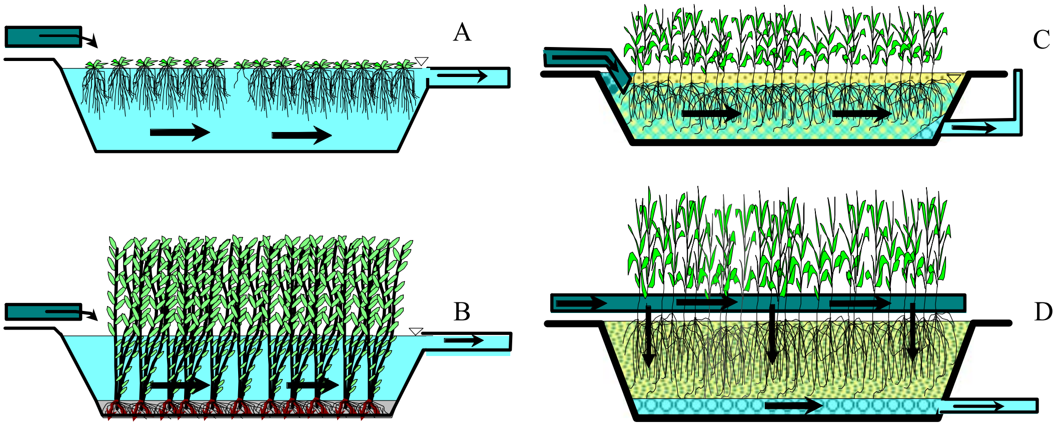

Figure 4 Pond/wetland systems for wastewater treatment (A, pond with free-floating plants; B, horizontal surface flow wetland or pond with emergent water plants; C, horizontal subsurface flow wetland; D, vertical flow wetland).

Treatment wetlands have some properties in common with facultative lagoons and also have some important structural and functional differences (see Figure 4A). Water column processes in deeper zones within treatment wetlands are nearly identical to ponds with surface autotrophic zones dominated by planktonic or filamentous algae, or floating or submerged aquatic macrophytes.

Horizontal surface flow wetland (see Figure 4B) consists of a shallow basin constructed of soil or other medium to support the roots of vegetation, and water control structure that maintains a shallow depth of water. The water surface is above the sediment, litter and soil, but live and standing dead plants parts are above water. This kind of wetland looks and acts much like natural marshes, and they can provide wildlife habitat and aesthetic benefits as well as water treatment.

In horizontal subsurface flow wetlands the water is fed in at the inlet and flows slowly through the porous medium under the surface of the bed in a more or less horizontal path until it reaches the outlet zone, where it is collected and discharged at the outlet (see Figure 4C). The wastewater will come into contact with a network of aerobic, anoxic and anaerobic zones. During the passage of the wastewater through the rhizosphere, the wastewater is cleaned by microbiological degradation and by physical and chemical processes (Brix 1987, Cooper et al. 1996). Whereas anaerobic processes predominate in subsurface flow system (apart from in the proximity of the helophyte roots), aerobic processes usually prevail in surface flow systems.

Vertical flow treatment wetlands (see Figure 4D) are composed of a flat bed of gravel topped with sand, with reeds growing at the same sort of densities as in horizontal flow system. They are fed intermittently. The liquid is dosed on the bed in a large batch, flooding the surface. The liquid then gradually drains vertically down through the bed and is collected by drainage network at the base. The bed drains completely free, allowing air to refill the bed.

The precise technology chosen has an important influence on the contaminant's biological degradation pathways and removal mechanisms. The mechanisms that are available in wetlands to improve water quality are therefore numerous and often interrelated. These mechanisms include:

- Settling of suspended particulate matter

- Filtration and chemical precipitation through contact of the water with the substrate and litter

- Chemical transformation

- Adsorption and ion exchange on the surfaces of plants, substrate sediment and litter

- Breakdown, and transformation and uptake of pollutants and nutrients by microorganisms and plants

- Predation and natural die-off of pathogens

2.2.6.2 Function of the plants

The macrophytes growing in constructed treatment wetlands have several properties in relation to the treatment processes that make them an essential component to the design. The most important effects of the macrophytes in relation to the wastewater treatment processes are the physical effects that the plant tissues give rise to (such as erosion control, filtration effect and provision of surface area for attached microorganisms). The macrophytes have other site-specific valuable functions, such as providing a suitable habitat for wildlife and giving systems an aesthetic appearance. The major roles of macrophytes in constructed treatment wetlands are summarized in.Table 1.

The general requirements of plants suitable for use in constructed wetland wastewater treatment systems include (Tanner, 1996):

- Ecological acceptability; i.e., no significant weed or disease risks or danger to the ecological or genetic integrity of surrounding natural ecosystems;

- Tolerance of local climatic conditions, pests and diseases;

- Ready propagation, and rapid establishment, spread and growth; and

- High pollutant removal capacity, either through direct assimilation and storage, or indirectly by enhancement of microbial transformations such as nitrification (via root-zone oxygen release) and denitrification (via production of carbon substrate).

Table 1 Summary of the major roles of macrophytes in constructed treatment wetlands (Brix 1987).

Macrophyte property	Role in treatment process
Aerial plant tissue	Light attenuation→ reduced growth of phytoplankton Influence on microclimate→ insulation during winter Reduced wind velocity→ reduced risk of resuspension Aesthetically pleasing appearance of system Storage of nutrients
Plant tissue in water	Filtering effect→ filter out large debris Reduce current velocity→ increase rate of sedimentation, reduces risk of resuspension Provide surface area for attached biofilm Excretion of photosynthetic oxygen→ increases aerobic degradation Uptake of nutrients
Root and rhizomes in the sediment	Provide surface for attached bacteria and other microorganisms Prevents the medium from clogging in vertical filter systems Release of oxygen increase degradation and nitrification Uptake of nutrients Release of antibiotics

The hydraulic retention times, including the length of time the water is in contact with the plant root, affects the extent to which the plant plays a significant role in the removal or breakdown of pollutants. Whereas plants significantly affect the removal of pollutants in horizontal subsurface systems with long hydraulic retention times used to clean municipal wastewater, their role is minor in pollutant removal in periodically loaded vertical filters, which usually have a short hydraulic retention time (Wissing, 1995).

The function of the plants in constructed wetland include gas transportation and release oxygen into the rhizosphere, uptake of inorganic compounds and release of carbon compounds.

2.2.6.2.1 Gas transport in the helophytes and oxygen release into the rhizosphere

It is well documented that aquatic macrophytes release oxygen from roots into the rhizosphere and that this release influences the biogeochemical cycles in the sediments through the effects on the redox status of the sediments (Barko et al. 1991; Sorrel and Armstrong, 1994).

The introduction of atmospheric air into the plant's interior means that under anoxic conditions a sufficient amount of oxygen (see Table 2) is available in the rhizome and root zones, which can be used for respiration. However, the oxygen transported in the airflow is also vital to the plant's survival in another respect.

Table 2 Oxygen release rates into the rhizosphere

Plants	Oxygen release rate			Author
	area specific rate: g O_2 $m^{-2}d^{-1}$	mg O_2 h^{-1} per plant	µmol O_2 h^{-1} g root dry mass	
Phragmites australis	0 – 0.9			Fruergaard et al., 1987
	0 – 30			Kramer, 1990
	5 – 12			Armstrong et al., 1990
	0.02			Brix and Schierup, 1990
Juncus effusus		0.91		Wießner et al., 2002
Typha latifolia			120 - 200	Jespersen et al., 1998
Juncus ingens			126	Sorrell and Armstrong, 1994

Oxygen is released into the rhizosphere and parts of the root system, mainly around the root tips and on young laterals (Armstrong et al., 1990; Flessa, 1991).

The release of oxygen causes the formation of an oxidative film directly on the root surface. This film protects the sensitive root areas from being damaged by toxic components like H_2S in the anoxic, usually extremely reduced rhizosphere (Armstrong et al., 1994; Vartapetian and Jackson, 1997). This protective film has a thickness of between 1 and 4 mm, depending on the way in which incoming oxygen-consuming wastewater flows against the roots, and it contains redox gradients ranging from about -250 mV, as frequently measured in reduced rhizosphere, to about +500 mV directly on the root surface (Flessa, 1991).

Root oxygen release rates from a number of submerged aquatic plants are reported to be in the range 0.5 – 5.2 g m^{-2} d^{-1} (Sand-Jensen et al. 1982; Kemp and Murray 1986; Caffrey and Kemp 1991) and from free-floating plants 0.25 – 9.6 (Moorhead and Reddy 1988; Perdomo et al. 1996). The wide range in these values is caused by species-specific differences, by the seasonal variation in oxygen release rates and by the different experimental technique used in the studies.

Plants can reduce the toxic effect of sulphide by preventing high oxygen loss along most of the root, especially at high sulphide concentrations, and allowing oxygen leakage only at the root tips and young roots, which are the most important parts for growth and nutrient up-take (Armstrong, 1971, 1979; Armstrong and Armstrong, 1988; Colmer et al., 1998; Connell et al., 1999; Končalová, 1990). Van der Welle et al. (2007) reported that oxygen loss from *J. effusus* roots was approximately five times higher than that from *C. palustris* roots, which likely resulted in the oxidation of sulphide (and iron) and decreased concentrations of the toxin. Moreover, *J. effusus* has a mechanism to prevent unnecessary oxygen loss along most of its roots by forming a layer of compact cells with thickened, lignified cell walls (Končalová, 1990; Kutschera and Lichtenegger, 1982), it should be able to transport oxygen to roots at greater dephts.

Root systems also release other substances besides oxygen (Seidel et al., 1966). It is also well known that a range of submerged macrophytes releases compounds that affect the growth of other species. However the role of this attribute in treatment wetlands has not yet been experimentally verified.

The aerenchyma tissue also plays a role in the methane emission through helophyte plants in wetlands which were estimated at 940 mg CH_4 $m^{-2}d^{-1}$ for a cattail wetland (Yavitt and Knapp, 1995). Thomas et al., (1996) summarized and cited other papers in which helophytes are responsible for 50-90 % of the total methane flux from wetlands. Tanner et al., (1997) estimated methane emission from constructed wetlands used to treat agriculture wastewater to account for around 2-4 % of wastewater carbon loads in vegetated wetlands and 7-8 % of loads in unvegetated systems.

2.2.6.2.2 Uptake of inorganic compounds by plants

Wetland plants require nutrients for growth and reproduction, and the rooted macrophytes take up nutrients primarily through their root system. Some uptake also occurs through immersed stems and levels from the surrounding water. Because wetlands plants are very productive, considerable quantities of nutrients can be bound in the biomass.

The main mechanisms of nutrient removal from wastewater in constructed wetlands are microbial processes such as nitrification and denitrification as well as physicochemical processes such as the fixation of phosphate by iron and aluminium in the soil filter. Moreover, plants are able to tolerate high concentrations of nutrients and heavy metals, and in some cases even to accumulate them in their tissues.

The mean phosphorus content in the dry biomass of a large number of helophytes was found to be around 0.15-1.05 % (McJannet et al., 1995). Consequently, less than 5 % of the phosphorous load in municipal wastewater is taken up by plants. Seen from this angle, the effect of harvesting the plant biomass is of low significance (Kim and Geary, 2001).

The uptake of nitrogen into the plant biomass is also of minor importance from a technical point of view since harvesting the aboveground biomass would remove only 5-10 % of the nitrogen (Thable, 1984). Tanner (1996) estimated the nitrogen concentration in helophytes in the aboveground biomass to be 15 and 32 mg N g^{-1} dry mass. Owing to these relatively low levels of nutrients, plant biomass is usually not harvested in Europe.

Sulphur compounds could be accumulating internally in plants as a result of reoxidation of sulphides. Holmer et al, (2005) reported that S^o was accumulating in eelgrass in the below-ground structure of the plants exposed to high sulphide concentrations with highest concentration in the youngest roots and oldest internodes. There was no accumulation of S^o in the leaves, suggesting that the intruding sulphide were reoxidized in the below-ground structures before reaching the leaves. The accumulation of S^o was higher in the roots of the low light treatment (up to two times) suggesting a large intrusion of sulphide.

The quantities of nutrients that can be removed by harvesting is generally insignificant in comparison with the loading into the constructed wetlands with the wastewater (Brix 1994; Geller 1996). If the wetlands are not harvested, the vast majority of the nutrients that have been incorporated into the plant tissue will be returned to the water by decomposition processes. Long-term storage of nutrients in the wetland systems results from the undecomposed fraction of the litter produced by the various elements of the biogeochemical cycles as well as the deposition of refractory nutrient-containing compounds (Kadlec and Knight, 1996).

From a technological point of view, the accumulation of heavy metals by plants is usually insignificant when industrial effluent and mine drainage are being treated. This is because the amount that can be accumulated is only a fraction of the total load of heavy metal in wastewater. Nevertheless, a number of terrestrial plants are known which can accumulate relatively high amounts of heavy metals in their biomass.

2.2.6.2.3 The release of carbon compounds from plants

Plants also release a wide range of organic compounds by roots (Rovira 1965, 1969; Barber and Martin 1976). The magnitude of this release is still unclear, but reported values are generally 5-25% of the photosynthetically fixed carbon. This organic carbon exudates by roots might act as a carbon source for denitrifiers and thus increase nitrate removal in some types of treatment wetland (Platzer 1994).

The entire process of carbon input is named as rhizodeposition. Rhizodeposition products (exudates, mucigels, dead cell material, etc.) cause various biological processes to take place in the rhizosphere. The quantity of organic carbon compounds released has been estimated at 10-40 % of the net photosynthetic production of agricultural crops in general because of the oxygen and carbon compounds donating helophyte roots a constructed wetland is a metabolically multi potent "technical ecosystem".

It is also conceivable that in zones of constructed wetlands with a low organic load, root exudates and dead plant material could be involved in the microbial cometabolic degradation of poorly degradable organic compounds (Moormann et al., 2002).

2.2.6.2.4 Elimination of pathogenic germs

The efficiency of germ elimination in constructed wetlands is subject to high fluctuation. There are positive examples with *Giardia* cysts, *Cryptosporidium* oocysts, total coliforms, fecal coliforms and Coliphages (Thurston et al., 1996). The examples demonstrate the potential of this technology, even if the mechanisms of germ reduction are not fully understood.

The very complex mechanisms in this system have so far only been studied to a limited extent. According to Ottova et al. (1997), important factors of influence in connection with reduction include the following:

- Physical: filtration, sedimentation, adsorption and aggregation;

- Biological: consumed by protozoa, lytic bacteria, bacteriophages, natural death;

- Chemical: oxidative damage, influence of toxins from other microorganisms and plants.

2.2.6.2.5 Physical effects

The presence of vegetation in wetlands distributes and decreases the current velocities of the water (Pettecrew and Kalff, 1992; Somes et al., 1996). This creates better condition for the sedimentation of suspended solids, decreases the risk of erosion and resuspension, and increases the contact time between the water and the plant surface area. The macrophytes are also important for stabilizing the soil surface in treatment wetlands, because their dense root systems impede the formation of erosion channels. In vertical flow systems the presence of the macrophytes, together with an intermittent loading regime, helps to prevent clogging of the medium (Bahlo and Wach, 1990). The movements of the plants, as a consequence of wind and other factors keep the surface open, and the growth of roots within the filter medium helps to decompose organic matter and prevent clogging.

The vegetation cover in a wetland can be regarded as a thick biofilm located between the atmosphere and the wetland soil or water surface in which significant gradients in different environmental parameter occur. Wind velocities are decreased near the soil or water surface in comparison with the velocities above the vegetation, which decreases the resuspension of settled material and thereby improves the removal of suspended solids by sedimentation. A drawback of decreased wind velocities near the water surface is, however, the decreased aeration of the water column.

Light is attenuates hindering the production of algae in the water below the vegetation cover. This property is used in duckweed-based systems, as algae die and settle out beneath the dense cover of duckweed (Ngo 1987). Another important effect of the plants is the insulation that the cover provides during winter, especially in temperate areas (Smith et al. 1996). When the standing litter is covered by snow it provides a perfect insulation and helps to keep the soil free of frost. The litter layer also helps to protect the soil from freezing during winter; however, it also keeps the soil cooler during spring (Brix 1994).

2.2.6.2.6 Other roles

The macrophytes in constructed treatment wetlands can have functions that are not directly related to the water treatment processes. In large systems, the wetlands vegetation can support a diverse wildlife, including birds and reptiles (Knight 1997, Worrall et al., 1996). This can be of importance, as natural wetlands and thereby wetland habitats have been destroyed at a high rate in many places. Another point that is perhaps most important in small systems serving, for example, single houses and hotel is the aesthetic value of the macrophytes. It is possible to select attractive wetland plants such as *Iris pseudacorus* (yellow flag) or *Canna* spp. (canna lilies) and in this way give the sewage treatment system a pleasant appearance.

2.2.6.3 Plants species

Constructed wetlands can be planted with a number of adapted, emergent wetland plants species. Wetlands created as part of compensatory mitigation or for wildlife habitat typically include a large number of planted species. However, in constructed wetland treatment systems, diversity is typically quite low.

The selection of plant species for wetlands (see Table 3) should consider the following variables: expected water quality, normal and extreme water depths, climate and latitude, maintenance requirements and project goals.

Table 3 Selection of plant species used in constructed wetlands (adapted from Stottmeister et al., 2003).

Scientific name	English name
Phragmites australis (Cav.) Trin. Ex Steud.	common reed
Juncus spp.	rushes
Scirpus spp.	bulrushes
Typha angustifolia L.	narrow-leaved cattail
Typha latifolia L.	broad-leaved cattail
Iris pseudacorus L.	yellow flag
Acorus calamus L.	sweet flag
Glyceria maxima (Hartm.) Holmb	reed grass
Carex spp.	sedges

At present there is no clear evidence that treatment performance is superior or different between the common emergent wetland plants species used in treatment wetlands. The best selection criteria are growth potential, survivability and cost of planting and maintenance. It is clear that densely vegetated areas are more effective at treating pollutants than are sparsely vegetated areas. A corollary to this observation is that plant species that provide structure year-round perform better than species that die below the water line after the onset of cold temperatures. For these reasons, fast-growing emergent species that have high lignin contents and that are adapted to variable water depths are the most appropriate for constructed wetland treatment systems. Wetlands plant genera that most successfully meet these criteria include *Typha*, *Scirpus* and *Phragmites* (see Table 3).

Many of the emergent species listed (sedges, *Juncus* sp. and grasses) were found growing in much deeper water in the constructed wetlands indicating their ability to adapt (or tolerate) to not only deeper water but also permanent water logging.

Plants are widespread, able to tolerate a wide range of environmental conditions, and can alter their environment in ways suitable for wastewater treatment. According to practical experiences and corresponding experiments, species of helophytes (marsh plants) work best of all in semi natural wastewater treatment systems. This is because helophytes possess specific characteristics of growth physiology that guarantee their survival even under extreme rhizosphere conditions. The extreme condition in the rhizosphere in wetlands used to treat wastewater can be summed up as following:

- Highly reduced milieu (Eh up to <-200 mV, especially in horizontal subsurface flow systems) prompting the formation of H_2S and CH_4;

- Acidic or alkaline pH values in certain wastewaters;

- Toxic wastewater components such as phenols, tensides, biocides, heavy metals, etc;

- Salinity

J. effusus is a perennial monocotyledonous species with an invasive character, which is often found on drained peatlands (Richards and Chapham, 1941). It generally has a high root porosity (~ 25-30 %), which is even increased under anaerobic conditions (Visser et al., 2000).

Tanner (1996) indicated that *Juncus effusus* shows the highest mean shoot density up to 4534 shoots m^{-2} of the eight tested species. Above-ground tissue nutrient concentrations were high but there was a low level of biomass production, and it was capable of growth in ammonium-rich organic wastewater, producing a compact stand without major seasonal die-back. It is unlikely to be competitive in mixed plantings growing in fertile wastewaters because of its low stature and productivity, but it may have potential for specialist applications, such as small single-dwelling treatment wetlands where minimal maintenance and low visual impact is sought.

Oranjewould (2000) found that while *Equisetum fluviatile* disappeared locally during the extreme dry summers, pH dropped from 7 to 3.5-3.2, and when sulphate concentration in surface water increased from 48 to 250 mg L^{-1}; other species like *Juncus effusus* and *J. acutiflorus* seemed to benefit from the temporary acid condition.

Kim and Geary (2001) showed that *Juncus effusus* and *Scirpus validus* have been trailed successfully in microcosms in North Carolina. The stems and leaves of emergent macrophytes and their roots, reduce water velocity and turbulence causing filtration and settlement of particles (sediment, organic particulates); and provide an increased surface area for attachment of epiphytic algae and microorganisms.

By the properties previously mentioned, *J. effusus* was selected as the species to work in this study; additionally, *Juncus effusus* is an evergreen plant which grows very well in advance of the frost-free period, especially spring–bloomers.

In order to understand more about the complexities of what happens when sulphide enters into the root zone, it is necessary to know more about the role of the *J. effusus* and other factors in the sulphide transformation reactions.

2.2.6.4 Microorganisms

Because of the presence of ample water, wetlands are typically home to a variety of microbial and plant species. The diversity of physical and chemical niches present in wetlands results in a continuum of life forms from the smallest viruses to the larges trees. This biological diversity creates interspecific interactions, resulting in greater diversity, more complete utilization of energy inflows, and ultimately to the emergent properties of the wetland ecosystem.

In constructed wetlands, the main role in the transformation and mineralization of nutrients and organic pollutants is played not by plants but by microorganisms. It has been shown that in the rhizosphere, the zone near the root cells, the density of microorganisms is higher than in the zone far from the roots.

Depending on the oxygen input by helophytes and availability of other electron acceptors, the contaminants in the wastewater are metabolized in various ways. In subsurface flow systems, aerobic processes only predominate near roots and on the rhizoplane (the surface of the root). In the zones that are largely free of oxygen, anaerobic processes such as denitrification, sulphate reduction and/or methanogenesis take place.

Nitrogen transformation in constructed wetlands has already been the subject of several papers. The main removal mechanism is microbial nitrification-denitrification; in contrast, incorporation into the plant biomass is only of minor importance (Cooper and

Maeseneer, 1996; Laber et al., 1999; Urbanc-Bercic and Bulc, 1994; Bayley et al., 2003).

Under aerobic conditions, ammonium is oxidized by micro-organisms to nitrate, with nitrite as an intermediate product. Two different groups of bacteria play a role in the nitrification step: ammonium oxidizers and nitrite oxidizers. In the oxidation of ammonia, nitrite is formed as an intermediate product. It has been considered that it can rarely be accumulated in terrestrial and aquatic environments. However, some reports indicate that nitrite can be accumulated in ecosystems.

An accumulation of nitrite was observed in pore waters of some estuarine sediments as well as in some treatment plants on a laboratory scale (Hanaki et al., 1990; Helder and de Vries., 1983), which was attributed to a lower affinity for oxygen of the nitrite oxidizers than of the ammonium oxidizers (Laanbroek and Gerards, 1993). Although a denitrification step of nitrite by electron donors like organic carbon, ammonia, H_2S, etc. is possible on this oxidation level, in treatment plants usually a total oxidation to nitrate is realized. With organic carbon, the denitrifiers reduce nitrate via nitrite to dinitrogen gas. In treatment plants, nitrate is reduced by the organic carbon load of a portion on "untreated" wastewater.

Recently, a new pathway was discovered by Mulder et al. (1995): Anamox bacteria can use nitrite as an electron acceptor and anaerobically convert ammonium and nitrite to nitrogen gas. In contrast to the traditional nitrification-denitrification route, Anamox is an autotrophic process. The microorganisms use bicarbonate as a carbon source.

Jackson and Myers (2002) reported that sulphate reducing bacteria were present throughout the free-water surface pilot wetland soil and water. The water chemistry suggested that conditions were well suited for these organisms to thrive in all parts of the wetlands. The high concentration of sulphate in the produced water ensured that there was a ready supply of substrate for sulphate reducing bacteria.

Less is known about the microorganisms involved in the S-transformation reactions in constructed wetlands.

2.2.6.5 Interrelation of microbial nitrogen and sulphur cycle

The major nitrogen transformation processes in wetlands are presented in Table 4. The various forms of nitrogen are continually involved in biochemical transformation from inorganic to organic compounds and back from organic to inorganic. All of these transformations are necessary for wetland ecosystems to function successfully.

Table 4 Nitrogen transformation processes in constructed wetlands

Process	Transformation
Volatilization	ammonia-N (aq)→ ammonia-N (g)
Ammonification	organic-N → ammonia-N
Nitrification	ammonia-N → nitrite-N → nitrate-N
Nitrate-ammonification	nitrate-N → ammonia-N
Denitrification	nitrate-N → nitrite-N → gaseous N_2, N_2O
N_2 Fixation	gaseous N_2 → ammonia-N (organic-N)
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N → organic-N
Ammonia adsorption	
Organic nitrogen burial	
ANAMOX (anaerobic ammonia oxidation)	ammonia-N → gaseous N_2

- The effect of sulphide and organic matter on the nitrification activity

Nitrification – the oxidation of ammonium to nitrate through nitrite may be carried out sequentially by the autotrophic bacteria *Nitrosomonas* sp. and *Nitrobacter* sp. The nitrification activity and bacterial growth rate is influenced by several environmental factors: pH, alkalinity, oxygen and ammonium concentration, temperature, organic matter concentration as well as occurrence of inhibitory compounds (e.g. sulphide).

Septic wastewater has been observed to have a negative impact on the nitrification activity in many treatment plants. However, there are a few references in the literature where reason and effects have been documented. Tomlinson and Bruce (1979) found that nitrification activity was reduced by 80% in an activated sludge plant when septic sewage was supplied, and that the nitrifying bacteria failed to establish when treating exceptionally septic sewage. The inhibition was avoided by pre-aeration of the sewage, indicating that the inhibitory substances could be sulphide and/or volatile organic compounds that are easily stripped. Unfortunately, there is no information given concerning the concentration of sulphide and organic matter. Beccari et al., (1980) found that nitrification activity was reduced by 28%, 67% and 76% at sulphide concentration of 1, 5 and 10 mg S L^{-1}, respectively. In biofilters it has been observed that the nitrification activity increased by about 10% as the concentration of sulphide was reduced from about 5.5 to 2 mg S L^{-1} (Bentzen et al., 1995). The extent of sulphide inhibition is supposed to be dependent on the composition of biomass, degree of acclimatisation, the concentration of sulphide, and the content of other inorganic and organic compounds in the wastewater. The toxic effect may be particularly harmful at low temperature because of the low growth rate of nitrifying bacteria ($\mu_{max} \sim 0.8\ d^{-1}$ at 20 ˚C, Henze et al., 1995).

- Simultaneous removal of nitrogen and sulphur under anaerobic conditions

The most conventional and thermodynamically favourable mechanisms involving sulphate and nitrogen compounds in anaerobic processes result in the formation of S^{2-} and NH_4^+. It was observed a significant production of molecular nitrogen resulting from the oxidation of TNK/ammonia and the simultaneous reduction of sulphate. Polanco et al., (2001) suggested that there might be a new degradation process wherein TKN/ammonia and sulphate are involved. Considering the most organic nitrogen is being transformed into ammonia by conventional ammonification process, Polanco et al. (2001) further considered ammonia as the only nitrogenous compound being oxidised to molecular nitrogen in this uncommon process. So far, the new discovered global oxidation-reduction mechanism is postulated in first approximation by following equation:

$$SO_4^{2-} + 2\ NH_4^+ \rightarrow S + N_2 + 4\ H_2O \qquad (2.12)$$

This global biochemical reaction could be obtained combining reactions involving nitrite formation and Anammox reaction:

$$3\ SO_4^{2-} + 4\ NH_4^+ \rightarrow 3\ S^{2-} + 4\ NO_2^- + 4\ H_2O + 8\ H^+ \qquad (2.13)$$

$$3\ S^{2-} + 2\ NO_2^- + 8\ H^+ \rightarrow N_2 + 3\ S + 4\ H_2O \qquad (2.14)$$

$$2\ NO_2^- + 2\ NH_4^+ \rightarrow 2\ N_2 + 4\ H_2O \quad (2.15)$$

- Denitrification by reduced sulphur compounds

For nitrogen, an important removal process is denitrification. Denitrification is the loss of nitrogen gas through the microbial reduction of nitrate to nitrogen. This process is controlled by the levels of oxygen, nitrate and organic matter (Seitzinger, 1990). Macrophytes, usually abundant in wetlands, offer ideal surfaces for the attachment and development of such biofilms (Wetzel, 1990).

Usually, either autotrophic or heterotrophic denitrification systems are used to remove nitrogen from wastewater. Heterotrophic denitrification is very efficient in terms of nitrate removal provided adequate amounts of organic carbon (Flere and Zhang, 1999; Zhang and Lampe, 1999). However, when organic carbon in the wastewater is insufficient compared to the nitrogen content, chemicals like methanol or similar organic compounds, must be added. For this reason, sulphur-based autotrophic denitrification has been receiving more attention recently due to two advantages: (1) there is no need for an external organic carbon source, like methanol or ethanol, which lower the cost and risk of the process; and (2) there is less sludge production, thereby minimizing sludge handling (Batchelor and Lawrence, 1978a; Claus and Kutzner, 1985; Koenig and Liu, 1996; Zhang and Lampe, 1999). However, autotrophic denitrification increases the sulphate concentration in the wastewater and consumes alkalinity.

Autotrophic denitrification system using sulphur-oxidizing bacteria oxidize reduced sulphur compounds (i.e., S^{2-}, $S_2O_3^{2-}$, SO_3^{2-}) as well as elemental sulphur to sulphate while reducing nitrate to nitrogen gas.

Considerable research has been conducted on sulphur based autotrophic denitrification (Gayle et al., 1989) including (1) the treatment of nitrate-contaminated groundwater (Flere un Zhang, 1999; Schippers et al., 1987; van der Hoek et al., 1992; Zhang and Lampe, 1999), (2) nitrate treatment in wastewater and landfill leachate (Koenig and Liu, 1996, 2001a), (3) the kinetic study (Batchelor and Lawrence, 1978a,b; Koenig and Liu, 2001b; Justin and Kelly, 1978) and (4) the effects of environmental conditions (i.e., aerobic or anaerobic) on sulphur/limestone autotrophic denitrification performance (Zhang and Lampe, 1999).

2.2.6.6 S-turnover in constructed wetlands

In fresh water wetlands, the degradation of organic sulphur from plants residues may result in higher sulphate concentration in the water column (Lefroy et al., 1994; Wind et al., 1995). The elevated sulphate concentration may then diffuse into the sediments where, in the presence of organic carbon provided by plants and in the absence of more energetically favourable terminal electron acceptors, organic carbon is degraded under sulphate reducing condition resulting in the build up of sulphide (Urban et al., 1994). Precipitates of iron monosulphide (FeS) and pyrite (FeS_2) are formed by the reaction of sulphide with iron compounds in the sediments (Berner, 1984; Howarth et al., 1984).

In the presence of metals, sulphide can form metal sulphide precipitates in the sediments and may control the metal concentration in the interstitial water (Boulègue et al., 1982; Emerson et al., 1983; Huerta-Diaz et al., 1998). Di Toro et al., (1990) observed that the most labile fraction of sediment sulphide, acid volatile sulphide (AVS), governs the trace metal bioavailability and hence their toxicity in the sediments. In addition to the solid phase AVS, sediment properties that influence partitioning behaviour of trace metals include organic carbon and iron and manganese oxides (Balistrieri et al., 1981; Bostick et al., 2001).

Precipitation of metal as sulphide rather than oxides has the following advantages:

- Alkalinity produced by sulphate reduction helps to neutralize acidity

- Sulphide precipitates are denser than oxide precipitates

- Sulphides are precipitated within the organic sediments and so are less vulnerable to disruption by sudden surges in flow.

Wetland plants have evolved specialized adaptations to survive in water saturated anoxic sediments. They transfer oxygen from the surface to the roots to support root respiration. A fraction of this oxygen diffuses into the surrounding sediments, where it can detoxify soluble phytotoxins (i.e. Fe^{+2}) and reoxidize reduced electron acceptors (i.e. Fe (II), Mn (II), NH_4^+, H_2S) formed during the degradation of organic matter in the anaerobic sediments (Armstrong, 1979; Dacey, 1980; Mendelssohn et al., 1995; Reddy et al., 1989; Sorrell, 1999).

Additionally, plants affect the biogeochemical dynamics of wetland sediment via evapotranspiration-induced advection, which increase the loading of dissolved constituents into the rizosphere (El-Shatnawi and Makhadmeh, 2001; Jaffé et al., 2001). Furthermore, plants release organic carbon into the sediments via litter, root exudates, and root turnover (Hale and Moore, 1979), which subsequently drive many biotic and abiotic reaction as the organic carbon in degraded and sediments become more reduced (Chanton and Dacey, 1991; Middelburg and Van Der Nat, 1998)

Physical transport processes and biogeochemical reactions, many of them driven by aquatic plants, may result in the extensive sulphur cycling between oxidizing and reducing conditions. Oxidation of sediment sulphide produces oxidized sulphur species (i.e. SO_4^{2-}, S^o) and may release associate metals to the water column (Simpson et al., 1998).

In constructed wetlands, especially subsurface horizontal flow systems, very little attention has been paid to the sulphur metabolism. 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 $m^{-2}d^{-1}$), Winter (1985) 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.

Until now processes which remove the environmentally problematic sulphur compounds from sewage are rare on a technical scale. The oxidation of reduced sulphur compounds in constructed wetlands opens a new possibility for wastewater treatment, especially because the constant release of oxygen in the rhizosphere is of particular interest in connection with the use of the rhizosphere to treat wastewater.

2.2.6.7 Sulphide toxicity to microorganisms

The toxicity of sulphide in anaerobic reactors has been well studied. Koster et al., (1986) reported that a free sulphide of 250 mg S L^{-1} caused 50 % inhibitions of methanogenesis in UASB granules. In a lactate-fed serum vial test, McCartney and Oleszkiewicz (1993) observed a 50 % inhibition of the methanogenic activity at 100 mg L^{-1} free sulphide. In an acetate-fed UASB reactor, a free sulphide of 184 mg L^{-1} was also found to cause a 50 % inhibition of methanogenesis at neutral pH (Visser et al., 1996).

The H_2S concentration is generally at its highest close to the sediment (Müller, 1966). The consequences for the biocoenosis are extremely severe since even at concentrations as low as 0.4 mg L^{-1} H_2S the fish toxic limit is reached (Liebmann, 1962).

2.2.6.8 Sulphide toxicity to plants

The presence of extensive aerenchyma system represents an important anatomical adaptation for transporting oxygen from above-ground organs to rhizomes and roots. Reduced gas flow in reed culms enhances anaerobic respiration, which results in a less efficient use of carbohydrates (Čížková-Kočnalová et al., 1992).

If organic matter accumulates and decomposes under anoxic conditions, phytotoxins are released into the soil.

In healthy sites, reeds are able to oxygenate the rhizosphere by convective flow through rhizomes of old dead culms (Armstrong et al., 1992), which may hence decrease concentration of sulphide in the rhizosphere. In contrast, severing rhizomes or clipping dead culm have been found to raise sulphide and ammonium levels in the soil, which led to decreased stem height, lower standing crop and reduce panicle size (Bart and Hartman, 2000).

Although sulphide may act as an inhibitor of N-uptake (Chambers et al., 1998; Mendelssohn and McKee, 1988), root absorption of both N and P did not seem to be hindered at die-back sites.

Sulphide may act as major phytotoxin, especially when environmental conditions such as waterlogged soil and high temperature affect gas diffusivity in roots and underground buds, eventually enhancing the entrance of phytotoxins into the plant. High sulphide concentration may lead to toxic effects to aquatic plants, such as root decay (root blackening and increased flaccidity of the roots) and mortality (Armstrong et al., 1996a; Smolder and Roelofs, 1996), reduced growth (Koch and Mendelssohn, 1989; Koch et al., 1990; Van der Welle et al., 2006) or even mortality (Lamers et al., 1998; Smolders et al., 1995).

Both sulphide and organic acids induce the formation of abnormal anatomical features such as callus blocking aerenchyma channels, lignifications and suberification of the surface layer of the root cells (Armstrong et al., 1996a; Armstrong and Armstrong, 1999). On the other hand, callus blockage can also be induced by insect damage (Armstrong et al., 1996a). It is known that sulphide is an inhibitor of aerobic respiration and nutrient uptake (Allan and Hollis, 1972; Mendelssohn and McKee, 1988).

Sulphide concentrations in sediment pore-water > 1 mM 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; Armstrong et al., 1996c). Additionally, Fürtig et al., (1996) found that energy metabolism in *Phragmites australis* is negatively affected even at sulphide concentration in pore-water as low as 1 mM.

The maximum concentration (mM) of some volatile monocarboxilic organic acids and sulphide found at die-back sites or in association with rotting underground parts of *Phragmites* for sulphide is 4 mM (128 mg L^{-1}) in die-back site, Lake Ferto, Hungary: Armstrong et al., (1996). (1.4 mM sulphide seriously damage *Phragmites* plants: Armstrong, 1999).

Goodman et al., 1995, found negative effects of sulphide on seagrass photosynthesis and increased mortality during die-back event have also been related to sulphide exposure (Carlson et al., 1994; Holmer et al., 2001). Intrusion of sulphide is considered to be the main cause for rapid die-back event of *Thalassia testudinum* in Florida Bay (Borum et al., 2005).

Van der Welle (2007) investigated the responses of the freshwater wetland species *J. effusus* L. and *Caltha palustris* to iron supply in sulfidic environments. *J effusus* showed a double advantage under sulphide-rich condition: it does not suffer from sulphide toxicity since it can oxidize potentially harmful reduced compounds in its rhizosphere and it can effectively profit from increased phosphate availability and overgrow or out-shadow other species.

Sulphide toxicity, however, can be mitigated by the formation of highly insoluble metal sulphides like iron sulphides (FeS, FeS_2 or pyrite) or metal sulphide complexes (Huerta-Diaz et al., 1998; Smolders and Roelofs, 1996; Wang and Chapman, 1999), thereby reducing both sulphide and metal toxicity. In areas where iron-rich groundwater is discharged, free sulphide concentration are usually low, as a result of iron sulphide precipitation.

2.2.6.9 Application of the technology

There are an expanding number of application areas for constructed wetlands technology. During the early years (1985) of the development of the technology, virtually all emphasis was on the treatment of domestic and municipal wastewater. Later the emphasis was on domestic wastewater, agriculture wastewater and mine drainage water (Mandi et al., 1998; Gearheart, 1992; Knight et al., 2000). In recent years there has been a branching to include a very broad spectrum of wastewater, including industrial and stormwaters. Increasing attention is now also being paid to using constructed wetlands to treat leachate, contaminated groundwater and industrial effluents.

There several roles for constructed wetlands in the treatment of domestic and municipal wastewaters. They can be positioned at any of several locations along the water quality improvement path. Constructed wetland technology is generally applied in two general themes for domestic and municipal wastewaters: for accomplishing secondary treatment and for accomplishing advanced treatment.

Constructed wetlands treatment systems can provide secondary treatment of domestic wastewater after mechanical pre-treatment consisting of a combination of screen, grit and grease chambers, sedimentation, septic and Imhoff tanks.

In recent years, constructed wetlands have been proving to be effective at treating acid mine drainage (AMD) (Hammer and Bastien, 1989; Klusman and Machemer, 1991). Early wetland designs treating coal mine drainage generally included peat and/or compost substrate, *Typha latifolia* (cattails), limestone gravel, and a surface flow system (Brodie, 1991; Brodie et al., 1988, 1989a,b; Calabrese et al., 1990; Eger and Lapakko, 1989; Hedin et al., 1988; Hiel and Kerins, 1988; Stark et al., 1988; Stillings et al., 1988). Recently, microbial sulphate reduction in wetlands has been used to treat acid mine drainage from coal mines in eastern U.S.A. (Dvorak et al., 1991; Hammack and Edenborn, 1991; Hammack and Hedin, 1989; Hedin et al., 1988, 1989; McIntire and Edenborn, 1990).

Early findings suggested that metal adsorption onto organic sites and microbial sulphate reductions with subsequent sulphide precipitation are important metal removal processes in the wetland (Wildeman and Laudon, 1989). Sulphate reducing bacteria were dominant throughout the wetland substrates, whereas significant populations of metal-oxidizing bacteria were only at the surface (Batal et al., 1989).

Further research indicated that anaerobic processes in the substrate lower Eh and sulphate concentration more effectively at lower mine drainage inflow rates (Wildeman et al., 1990). This led to the limiting reactant concept for a sulphate-reducing treatment system. At higher inflow rates, sulphide is the limiting reactant for metal sulphide precipitation, and this causes lower pH values, higher Eh values, and inconsistent metals removal (Reynolds et al., 1991). Alternatively, at lower inflow rates, dissolved metals are the limiting reactant for metal sulphide precipitation, and the excess sulphide and HCO_3 ensure higher pH values, lower Eh values, and consistent metals removal.

Additional data have also indicated that sulphate reduction coupled with sulphide precipitation is a more important metal removal process after the initial start up of the wetland than the adsorption of metals onto organic material in substrate (Machemer and Wildeman, 1992). Because of the importance of sulphate reduction in the wetland, Reynolds et al, (1991) determined an average maximum sulphate reduction rate for the wetland using a serum bottle experiment. The rate ranged from 0.5 to 1.2 μmol $g^{-1}d^{-1}$ of dry substrate.

2.2.6.10 Engineering aspects of treating sulphide containing wastewater

- Wastewater treatment plant including an anaerobic digestion step

In many tropical countries, anaerobic digestion of effluents is the main treatment step for wastewater treatment. The main advantage is that this treatment needs only small amount of external energy (electricity) supply for running reactors, etc. Figure 5 shows a process flow diagram of a wastewater treatment plant consisting of anaerobic treatment.

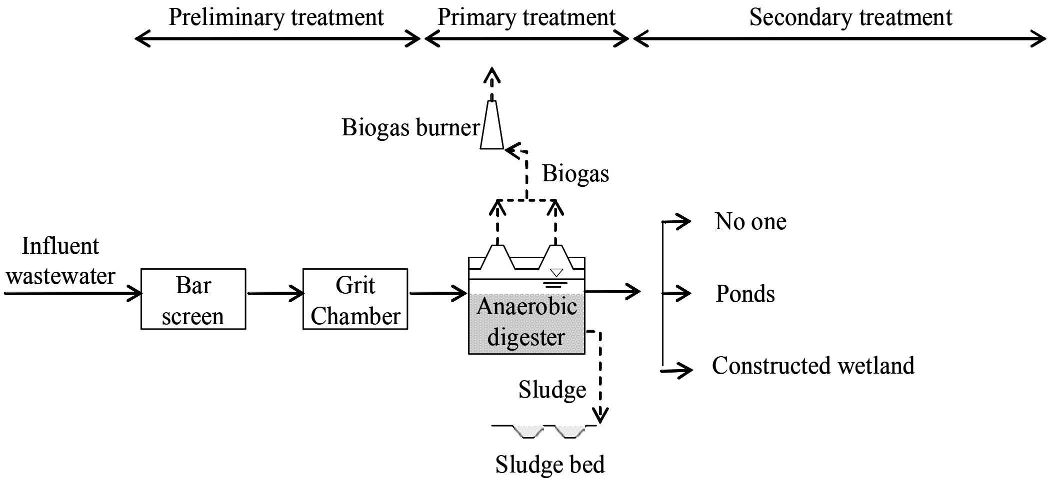

Figure 5 Process flow diagram for a treatment plant designed to meet secondary treatment with constructed wetland.

The first step in wastewater treatment is the removal or reduction of coarse solids. The usual procedure is to pass the untreated wastewater through bar racks or screens. Grit chambers are designed to remove sand, gravel, cinder, or other heavy solid materials that have subsiding velocities substantially grater than those of the organic putrescible solids in wastewater.

Primary treatment in septic tanks, anaerobic filter, UASB, has been applied successfully for domestic wastewater. In many tropical countries like Columbia the objective of this treatment is to remove mainly organic carbon of the wastewater. In this process biosludge is produced (about 90 % less than in the aerobic activated sludge process) and its excess is dried on sludge beds. After the material is dried, depending on its content of contaminants like heavy metal either it can be used as a soil improver or it has to be deposited in a dump.

In the primary treatment (anaerobic digester) biogas is produced, this might be captured and used as a source of energy. The emission of unburned biogas is associated with two negative effects: the release of green house gasses, methane and nitrous oxide; and H_2S with its obnoxious odour influencing the area downwind from the plant.

Because of the poor effluent quality of the anaerobic digestion an additional post-treatment is highly needed. This is realized in ponds and constructed wetlands.

- Source of sulphur compounds in municipal wastewater

Many compounds have been identified in sewage treatment works odours (see Appendix C). Typically, these compounds are reduced sulphur or nitrogen compounds, organic acids, aldehydes or ketones.

Domestic sewage typically contains 3-6 mg L^{-1} organic sulphur, derived mainly from proteinaceous material and can contain further organic sulphur (about 4 mg L^{-1}) resulting from sulphonates used in household detergents (Boon, 1995). Inorganic sulphur, in the form of sulphate, is present in quantities depending on the hardness of the water, typically in concentrations of 30-60 mg L^{-1} (Boon, 1995; Cheremisinoff, 1988). Considerably higher concentrations of sulphate may result from infiltration water or industrial sources (Harkness, 1980).

- Odour sources in wastewater treatment plants

Frechen (1988) identified the major odour sources at 100 German sewage treatment works by means of questionnaires completed by the works operators. Although a somewhat subjective method, the results (see Figure 6) reinforce the general opinion that the major odours sources are associates with inlet works, primary sedimentation tanks or sludge processing.

A main odour sources in a sewage treatment works is the inlet system. The incoming sewage still contains a high load of organic carbon compounds with sludge where anaerobic condition results in the formation of new odorants.

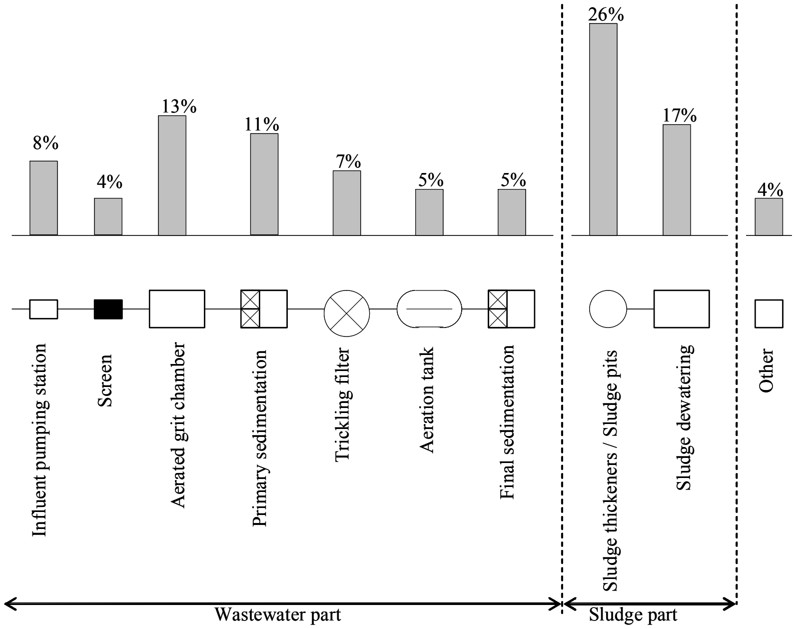

Figure 6 Major sources of odour identified from Frechen's (1998) survey of 100 German sewage treatment works (Figures are percentage of respondents who identified the process as an odour source).

In some cases, both conditions are satisfied. Primary tanks, for example, will promote the emission of odorants formed in the sewerage system by virtue of large area of sewage which is contacted with the atmosphere, and also by means of the turbulence generated at the inlet or outlet weirs. Where desludging is infrequent, large volumes of sludge accumulate and anaerobic conditions develop within the stored sludge. This can lead to the formation of new odorants. Primary tanks have been identified as major sources of odour at many works, especially where the influent sewage is septic and where desludging is infrequent (Hobson, 1995; Vincent and Hobson, 1988).

Aerobic treatment tends to reduce liquid-phase odorant concentrations due to biological oxidation. It can, however, be significant odour source as they bring large amounts of sewage into contact with air and can promote the stripping of odorants especially if overloaded (Vincent and Hobson, 1988). Aerobic treatment can be also a major source of volatilisation of non-domestic odorants, such as petrochemical or solvents (McGovern and Clarkson, 1994).

- *Odour and H_2S generation in anaerobic wastewater treatment*

Sulphate may be present in municipal sewage due to collection of industrial wastes rich in this anion or to natural content in water supply. Hydrogen sulphide is produced in an anaerobic environment mainly by sulphate reduction.

Further odorous organic compounds that has been found in wastewater treatment plants are carbon oxysulphide (COS), carbon disulphide (CS_2), mercaptanes of low molecular weight (R-SH), thiophenes (C_4H_4S), dimethylsulphide ($(CH_3)_2S$), dimethyldisulphide ($(CH_3)_2S_2$) and dimethiyltrisulphide ($(CH_3)_2S_3$), (Allen and Phatak, 1993). Other odorous molecules include mercaptans, ammonia, inorganic and organic amines, organic acids, aldehydes and ketones. In this environment, H_2S possesses such characteristic odour that it generally masks the scent of other organic sulphide compounds (Bhatia 1978; Smet and Van Langenhove 1998). For this reason, H_2S is the most characteristic bad odour constituent in biogas and in the environment of anaerobic digesters and wastewater treatment facilities in general (Carlson and Leiser 1966; Cho et al. 1992; Allen and Phatak 1993; Fernandez-Polanco et al. 1996; Martínez and Zamorano 1996; Metcalf and Eddy 2005). In fact, many research works on odour control consider H_2S as the reference compound.

Hwang et al., (1995) have analyzed the influent wastewater in a study of malodorous substances in wastewater at different steps of sewage treatment (seeTable 5). Although the results in Table 5 only represent examples of the odorous compounds shown in Appendix C, they may appear in wastewater in relative high concentrations.

Table 5 Sulphur and nitrogen containing odorous compounds in the influent wastewater at a treatment plant (Hwang et al. 1995).

Compound	Average concentration, (μg l^{-1})	Range of concentration, (μg l^{-1})
Hydrogen sulphide	23.9	15 - 38
Carbon disulphide	0.8	0.2 - 1.7
Methyl mercaptan	148	11 - 322
Dimethyl sulphide	10.6	3 - 27
Dimethyl disulphide	52.9	30 - 79
Dimethylamine	210	-
Trimethylamine	78	-
n-propylamine	33	-
Indole	570	-
Skatole	700	-

It should be noticed that the concentrations are those observed in wastewater. What appears in the air phase depends on a number of characteristics for emission.

The detection of H_2S become detectable in concentration as low as 0.008 ppm (see Appendix D). The concentration of H_2S found in treatment plants can vary considerably depending on the type of processes involved and on the characteristics of the wastewater. In this sense, Rands et al. (1981) found H_2S concentration in municipal treatment works between 45 and 537 part per million per volume (ppmv) and up to 1000 ppmv in the biogas from anaerobic sludge digesters. Lang and Jager (1992) and Webster et al. (1996) reported concentration of H_2S between 0.1 and 10 ppmv. Other compounds associated with odours in wastewater facilities are dimethylsulphide and methyl mercaptan. Cho et al. (1992) and Allen and Phatak (1993) found these volatile organic compounds (VOCs) at concentration between 5 and 40 ppmv.

The ratio of the three sulphide species (H_2S, HS^-, and S^{2-}) in water is pH dependent. The relative distribution of the three species, as a function of the pH, is presented in Figure 7.

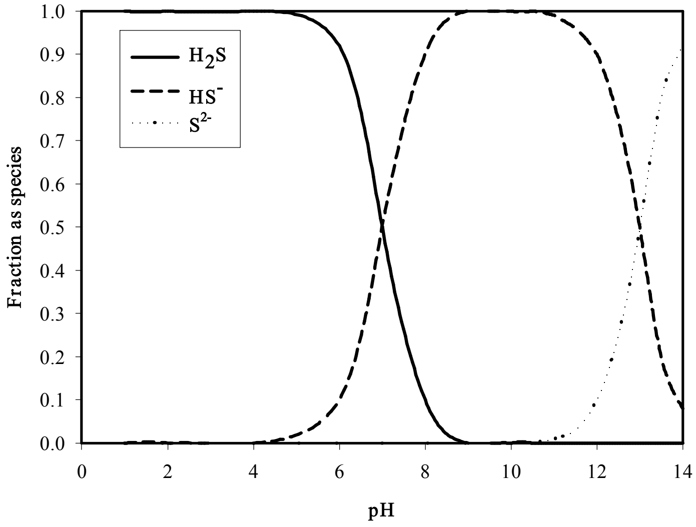

Figure 7 Relative ratio of hydrogen sulphide, bisulphide and sulphide in dependence on pH.

As can be seen, the amount of the high volatile H_2S is very sensitive to pH. At pH ≤ 5.5, almost all sulphide is H_2S ($\geq 97\%$) while at pH ≥ 8.5, less than 3% is present as H_2S. At pH around 7, the common operational value in anaerobic wastewater treatment, H_2S and HS^- will be present in solution close to an equal ratio (50% for each).

A moderate (0.5 mg L^{-1} or greater) liquid sulphide concentrations can result in high vapour-phase H_2S concentration. For example, at 20°C, wastewater discharging from a source containing a sulphide concentration of 1 mg L^{-1} at a pH of 7 would generate vapour-phase H_2S concentration of the order of 150 ppmv (Witherspoon et al., 2004). Although equilibrium condition are not generally achieved, values of 20 % to 50 % of equilibrium (or 20 to 75 ppmv H_2S) are routinely measured at these turbulent locations. Further, given that detection of H_2S occurs at concentration of approximately 1 ppbv, these locations have the potential for significant odour generation.

- Possibilities for limiting the sulphide intake to constructed wetlands and intensification of H_2S oxidation

The search for alternative solutions related to wastewater treatment systems has taken advantages of some favourable environmental conditions that amplify the range of applications of non-conventional systems. This is the case of the anaerobic processes for wastewater treatment in tropical countries and due to rising energy cost in an increasing extent also in other countries.

At the moment, there are some well established technologies successfully applied for domestic sewage treatment that have anaerobic reactors as the core of the system. The alternatives for the post-treatment of anaerobic effluents based on soil/plants system such as constructed wetlands are recognised as one of the technologies that can be used in conjunction with or as an alternative to anaerobic effluents (Mbuligwe, 2004).

Numerous studies have indicated that primary settled domestic wastewater subjected to horizontal subsurface-flow constructed wetlands treatment will typically experience reduction in the loading of thermotolerant coliform by 2-3 log-units, of total suspended solid (TSS) by up to 90%, 5-day biochemical oxygen demand (BOD_5) by up to 95% and total nitrogen (TN) by up to 70% (Kadlec and Knight, 1996; Davison et al., 2005). Nevertheless, the sulphide loading has not been considered as a factor that can disturbed the operational performance.

To overcome the problem of the sulphide intake to subsurface-flow wetlands, different approaches are possible (see Figure 8).

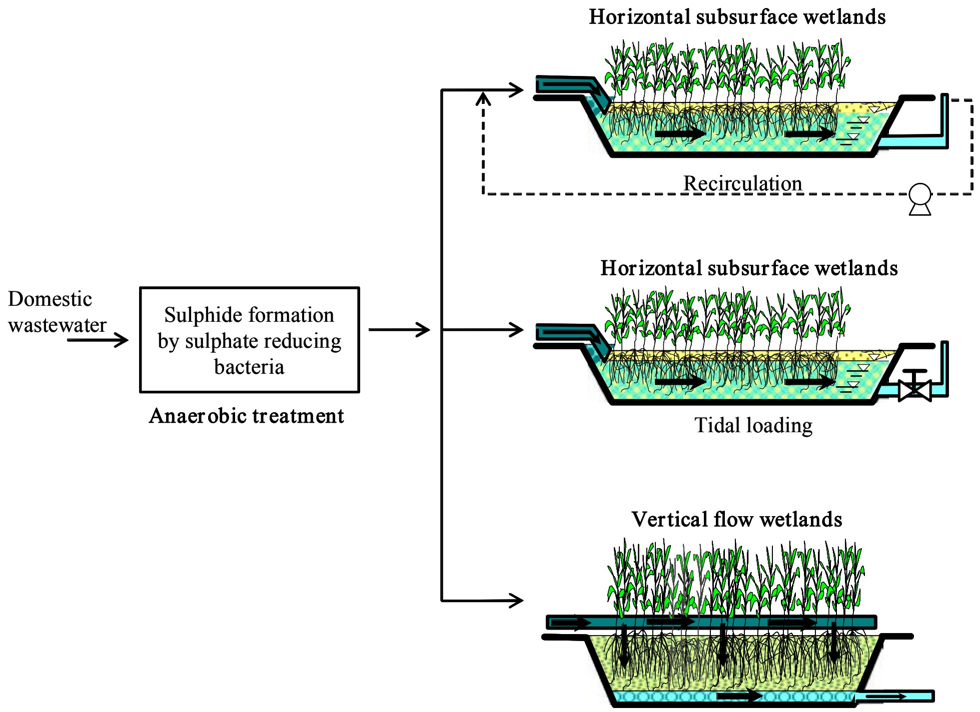

Figure 8 Possibilities for limiting the sulphide intakes to constructed wetlands.

The subsurface wetland with effluent recirculation flow allows decreasing the inflow sulphide loading. By this toxicity effects to plants can be minimized.

The subsurface horizontal flow wetland with "tidal loading" refers to an operation that repeatedly allows wetland soil pores to be filled with wastewater, and then completely drained. When the matrices are filled, maximum pollutant-biofilm contact is established. As the wastewater drains, air is drawn from the atmosphere into the matrices, to replenish the biofilms with oxygen. Through the artificial cycle of 'wet' and 'dry' periods, the wastewater acts as a passive pump to draw oxygen in to the wetland. Thus, the tidal loading operation has the potential of improving treatment efficiency through extended H_2S oxidation, enhanced aerobic microbial decomposition and pollutant-biofilm contact.

Vertical flow is also considered as an alternative to overcome the problem of the sulphide intake to wetlands. Forced aeration by intermittent loading and vertical draining increased local microsite oxygen diffusion rate by three orders of magnitude above reported oxygen exudation from plant roots (Lemon et al., 2003). Regarding sulphide removal, Giraldo (2001), pointed out that vertical flow wetlands removed sulphides at loads up to 20 g S^{2-} $m^{2-}d^{-1}$.

3 Material and Methods

3.1 Characterization of sulphide toxicity to *Juncus effusus*

The experiments presented here were conducted to investigate the levels of sulphide concentrations that cause toxicity effects to *J. effusus*.

3.1.1 Plant material

Plants of *Juncus effusus* from the greenhouse were taken for this study. After separating and choosing the plants, presenting homogenous and morphological traits, they were placed in the phytotechnical laboratory operating under defined environmental condition with a temperature day/night 22/16 °C; light day > 10 klux; day/night 16/8 hours.

3.1.2 Experimental set-up

Plants were placed into 7 flasks (300 ml-Erlenmeyer) with about 5 plants each (Figure 9) for 50 days. At first the plants were acclimatised for 25 days to the new environment (phytotechnical laboratory) and placed with their roots in a nutrient solution (tap water with a commercial fertilizer, Hakaphos at a concentration of 1 g L^{-1}). During this acclimatization period (25 days) plants were well adapted and no visual indication of plant stress was found.

After this acclimation period the toxicity test to sulphide started. By this the plant roots were exposed in a solution composed of tap water with 10 mM Tris/HCl buffer and 100 mg L^{-1} NaEDTA (to realize a constant pH of 7.2 ± 0.5 and keep trace metals of the tap water in the solution) to which different amounts of sodium sulphide ($Na_2S \cdot 9H_2O$) were added (0, 5, 10, 25 and 50 mg S^{2-} L^{-1}). Because of the high reactivity of sulphide with oxygen and to keep stable sulphide concentrations of these solutions, every day the old solutions were replaced by freshly new prepared solutions.

Figure 9 Experimental set up of the sulphide toxicity test of *J. effusus* after 50 days.

3.1.3 Measurements of plant related parameters

3.1.3.1 Water uptake

The evapotranspiration of the flasks with the plants and control (without plants) was estimated by weighting each flask every 24 hours. The difference between evapotranspiration and the evaporation (control without plants) allowed the calculation of the water uptake.

Fresh plant biomass was estimated by weighting each single plant before and after 24 hours. As indicator of plants growth the ratio between the water loss and the fresh biomass was used.

3.1.3.2 Relative growth rate

The length of all shoots in each flask was measured, providing total shoot length per plant as an indicator of above-ground biomass. These data allowed for calculation of relative growth rates (RGR) using the equation:

$$RGR = (\ln W2 - \ln W1) / t2 - t1 \qquad (3.1)$$

Where W_1 and W_2 are non destructive estimates of biomass shoot length for times t_1 (beginning of period) and t_2 (end of period), respectively (Beadle, 1982).

3.1.3.3 Chlorophyll *a* fluorescence

The measurements were made using a portable chlorophyll-fluorescence meter (PAM-2000, Walz). Three mature healthy shoots per flask were randomly chosen. A small leaf-clip adapter was placed on the central part of each shoot for 15 min to achieve dark pre-adaptation before determining *F*o, the fluorescence of photosystem II in the fully oxidized state, and *Fm*, the fluorescence following a pulse of saturation light. *Fv* (*Fm-Fo)* and *Fv*/*Fm* data are calculated on-line and recorded in a data file. *Fv*/*Fm* values are used to assess the photochemical efficiency of photosystem II (Krause and Weis, 1991).

3.1.3.4 Visual observation

Each plant was daily checked. Stress symptoms of *J. effusus* were noticed by a general yellowing (chlorosis) of older shoots. Frequently this yellow shoots turned to brown (necrosis). A “die-back” response was defined by visual loss of green colour (chlorosis) and necrotic symptoms as indicator of plants death.

3.2 Treatment of sulphide containing model wastewater in the Planted Fixed Bed Reactor

3.2.1 Synthetic wastewater

In this work the alternative for the post-treatment of anaerobic effluents was investigated under conditions of subsurface horizontal flow constructed wetlands; operation, temperatures of tropical countries were taken into account. Moreover, the characteristics of the model wastewater (see Table 6) were very close to that of a typical anaerobic digester effluent of domestic wastewater. It contained sulphate and sulphide simultaneously. Figure 10 shows the mean values of the anaerobic digester effluent of Bucaramanga-Colombia.

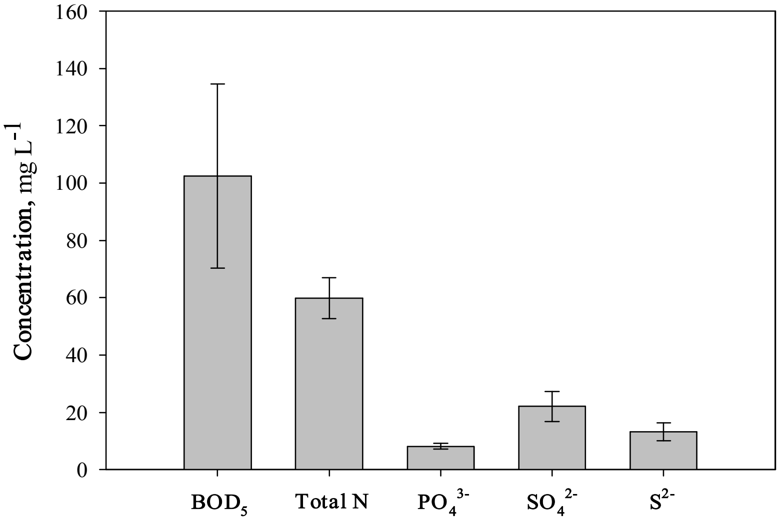

Figure 10 Effluent characteristics of the UASB-reactor of the Bucaramanga treatment plant (Colombia). (Bars are shown with standard error of the mean (n = 25, Feb-Nov-2004).

The artificial wastewater simulated an effluent of an anaerobic wastewater treatment plant with a calculated BOD$_5$ of about 100 mg L^{-1} derived from a carbon source (acetate) of good bioavailability (see Table 6).

Table 6 Chemical composition of the synthetic wastewater

Compound	Concentration (mg L^{-1})
CH_3COONa	128
NH_4Cl	148
NaH_2PO_4	12
NaCl	7
$MgCl_2x6H_2O$	1.64
$CaCl_2x2H_2O$	4
$Na_2S \cdot 9H_2O$	5
Trace mineral solution (see Table 7)	1 ml L^{-1}

The composition of the trace mineral solution is shown in the Table 7.

Table 7 Chemical composition of the trace mineral solution

Compound	Concentration (mg L^{-1})
EDTA-Na	100
$FeSO_4 \cdot 7H_2O$	100
$MnCl_2 \cdot 2H_2O$	80
$CoCl_2 \cdot 6H_2O$	170
$CaCl_2 \cdot 2H_2O$	70
$ZnCl_2$	100
$CuCl_2 \cdot 2H_2O$	150
$NiCl_2 \cdot 6H_2O$	30
H_3BO_3	10
$Na_2MoO_4 \cdot 2H_2O$	10
$Na_2SeO_3 \cdot 5H_2O$	2
HCl	3 ml L^{-1}

These compounds were dissolved in tap water with a sulphate concentration of about 150 mg S L^{-1} during the experimental phase A and in deionised water with a sulphate concentration of about 1.4 mg S L^{-1} during the experimental phases B and C. The resulting BOD_5, nitrogen and phosphorous ratio was of about 10:5:1.

To minimise abiotic sulphide oxidation in the storage tank the artificial wastewater was initially purged with nitrogen gas for at least 30 minutes to strip out dissolved oxygen. This reduced the dissolved oxygen concentration to approximately 0.1 mg L^{-1} (Yongsiri et al., 2003). Sodium sulphide ($Na_2S{\cdot}9H_2O$) was then added to the water phase. The headspace of the storage tank was kept oxygen-free by continuously purging of nitrogen gas.

Despite of the nitrogen atmosphere in the feeding tank the sulphide concentration was not stable resulting in a sulphide concentration in a range of 0.92 – 1.4 mg S L^{-1}. Some thiosulphate existed already as an impurity of the sodium sulphide respectively was formed from sulphide by autoxidation during storage. That is why the artificial wastewater was prepared every 3 days anew.

Figure 11 shows mean sulphurs species (sulphate-S, sulphide-S, sulphite-S and thiosulphate-S) in the feeding tank.

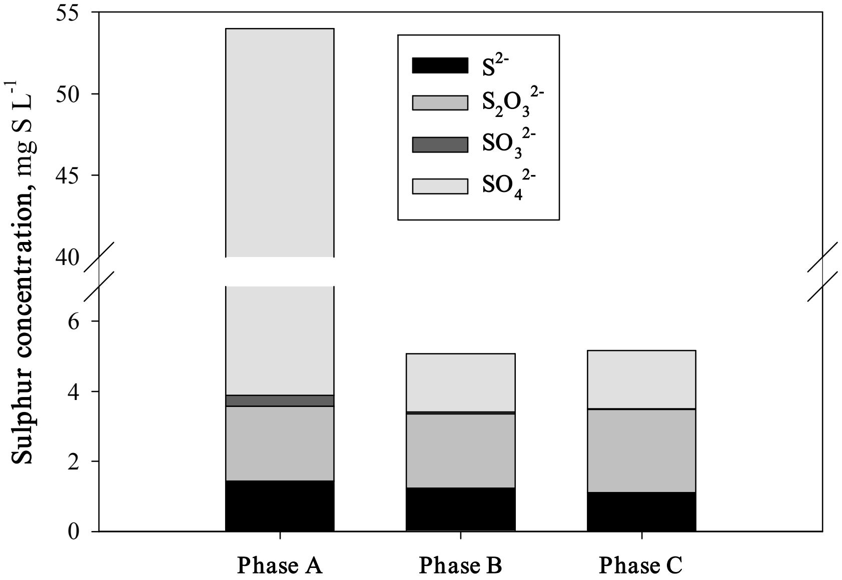

Figure 11 Sulphur species in the feeding tank of the Planted Fixed Bed Reactors.

3.2.2 Laboratory-scale reactor

Two laboratory-scale model wetlands were performed under condition of complete mixing of the filter bed by permanent circulation of the pore water. Since the internal flow conditions are comparable to an ideal mixed vessel, macro-scale gradients of concentration, Eh, pH, etc. were equalized and the effects of micro-gradient changes could be determined. The design and the principles of operation of the reactors were previously described in detail (Kappelmeyer et al., 2002; Wiessner et al., 2005a).

Figure 12 shows the scheme of the Planted Fixed Bed Reactor -PFBR. The reactor consisted of a PVC vessel with 30 cm in diameter and 30 cm tall. A basket of perforated stainless steel 28 cm in diameter and 30 cm tall was placed centrally inside the vessel. A pipe of the perforated stainless steel with 4.5 cm in diameter and 30 cm tall was placed centrally inside the basket. The basket was completely filled with gravel around the pipe. The reactors were closed with a lid containing eight holes through which the plants (18 shoots per hole) in the gravel bed grew. The gravel beds were 28 cm and the water levels were adjusted to 2.5 cm below the surface of the gravel beds.

The circulation flow was adjusted to 10 times the inflow. This permanent mixing of the process fluid made for hydrodynamic condition similar to an ideal mixed vessel inside the rhizosphere (Kappelmeyer et al., 2002).

The water level in the reactor was controlled by a sensor, which is controlled for the internal recycling system. The recirculation system is connected to a microprocessor Standard (WTW, pH-Ionen-to Put pMX 3000/pH) that permits on line measurement of the pH and the redox potential (Eh) recorded every 20 minutes.

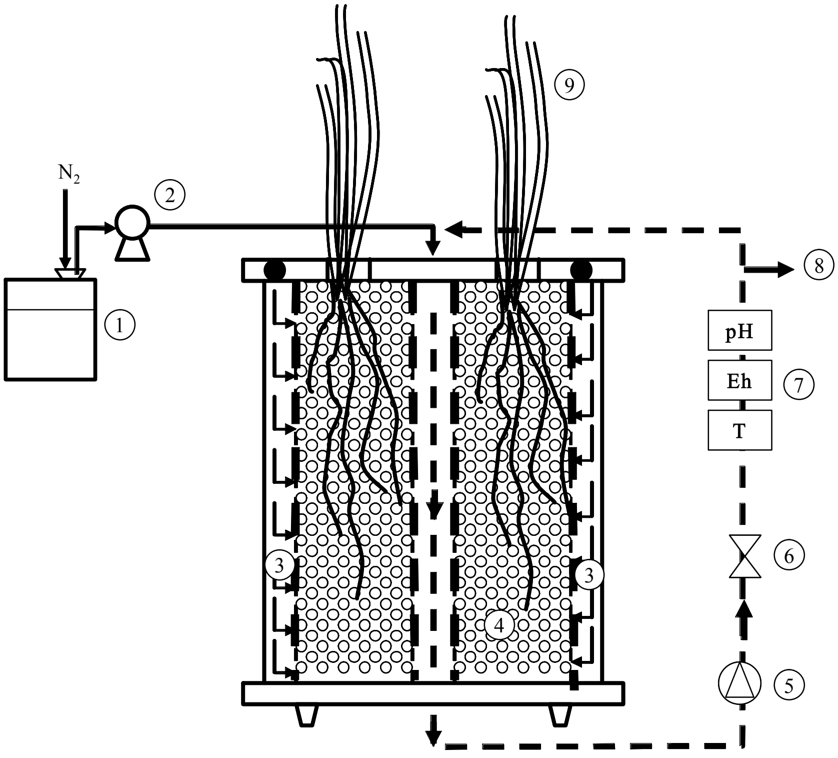

Figure 12 Diagram of the Planted Fixed Bed Reactor- PFBR (1 Feeding storage tank, 2 Pump, 3 Distribution chamber, 4 Gravel bed, 5 Recirculation pump, 6 Magnetic valve, 7 On line measurement , 8 Outflow , 9 Plants) (adapted from Kappelmeyer et al., 2002).

The physical and operational characteristics of the PFBR are shown in Table 8.

Table 8 Physical and operation characteristics of the PFBR

Characteristics	
Soil material	Gravel
Gravel diameter ,mm	2 – 8
Uniformity coefficient of the gravel, D_{60}/D_{10}	1.1
Porosity, %	0.42
Total reactor volume, L	20.1
Effective reactor volume, L	13.2
Height of the reactor, mm	280
Flow rate, ml min^{-1}	0.9 - 1.8
HRT, d	5 - 10
Circulation factor ,$V_{circulation}/V_{inflow}$	10

3.2.3 Plants biomass

Juncus effusus plants were pre-grown in hydroponic culture under greenhouse condition at a temperature of 25 °C. After choosing plants, presenting homogenous morphological traits, they were transported to the phyto-technical laboratory (temperature day/night 22/16 °C; light day > 10 klux; day/night 16/8 hours). During the acclimatization period of 1 week, the plants were fed with tap water and fertilized (NPK, Hakaphos) at a concentration of 1 g L^{-1}.

Both PFBR were planted with macrophytes (*J. effusus*). A density of 6978 and 9973 shoots m^{-2} for PFBR1 and PFBR 2 respectively were achieved.

3.2.4 Experimental conditions

The Planted Fixed Bed Reactors were run under three different conditions (phases A, B and C) realised by different sulphate concentrations in the feeding tank and different hydraulic loading rates (see Table 9).

Table 9 Operation conditions (phases A, B and C) of the Planted Fixed Bed Reactors realised by different sulphate concentrations and different hydraulic retention times of the artificial wastewater.

Parameter	Phase		
	A	B	C
Hydraulic retention time, d	5	5	10
Sulphide concentration, mg S L^{-1}	5	5	5
Sulphate concentration, mg S L^{-1}	50.1	1.4	1.4
Hydraulic loading rate, m d^{-1}	0.08	0.08	0.04
Organic loading rate, g BOD_5 m^{-2} d^{-1}	5.2	5.1	2.6

The experimental wetlands (see Figure 13) were placed in a greenhouse operating under defined environmental conditions with a temperature of 16-22°C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The experiments were run from middle of September 2004 to April 2005.

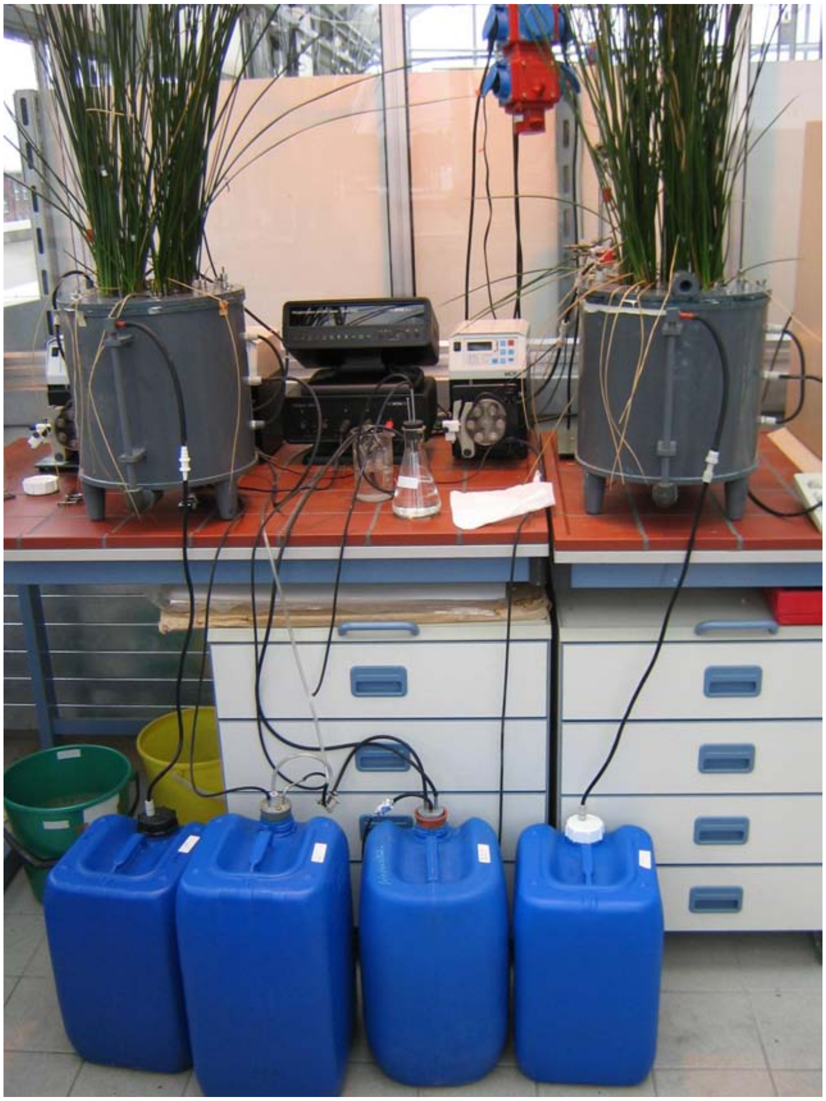

Figure 13 Experimental set up of the Planted Fixed Bed Reactor -PFBR

3.2.5 Sampling

Water samples were taken from the inlet and outlet zones of each bed (PFBR 1 and PFBR 2) with a syringe and a long needle which was rinsed in advance with N_2 gas to minimise autoxidation of sample ingredients.

3.3 Treatment of a sulphide containing model wastewater in the Laboratory-scale Horizontal Subsurface Flow Wetland

3.3.1 Synthetic wastewater

At the beginning of the experiment the wetland-scale reactors were fed with tap water to which was added trace mineral solution(1ml L^{-1}, see Table 7) and nutrient salts (Hakaphos, 0.1 g L^{-1}).

Artificial wastewater (see 3.2.1) simulating an effluent of an anaerobic wastewater treatment plant with acetate as carbon source was prepared in deionised water. Sodium sulphide ($Na_2S\cdot 9H_2O$) to reach 5 and 15 mg S L^{-1} was added into the feeding tank resulting in the mean sulphur species, which are shown in.Figure 14.

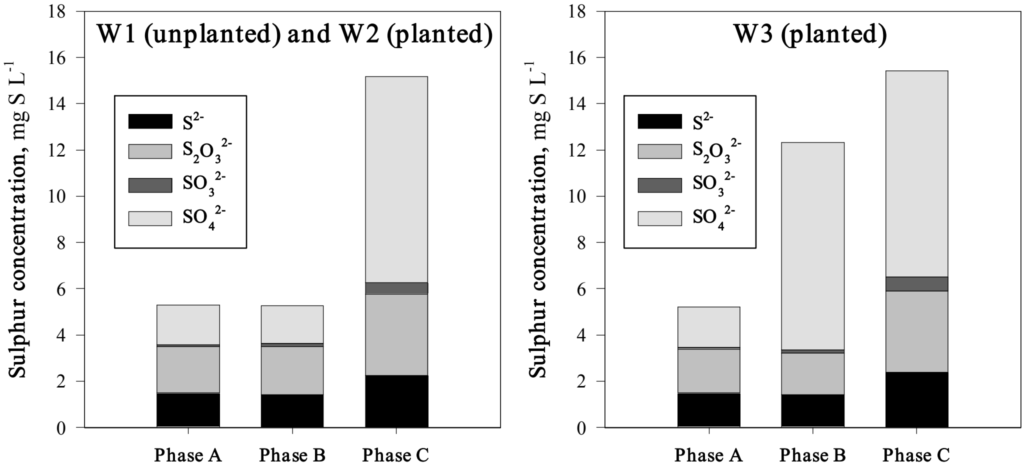

Figure 14 Sulphur species in the feeding tank of the subsurface horizontal laboratory-scale constructed wetland.

3.3.2 Laboratory-scale reactor

The diagrammatic sketch of the used laboratory-scale subsurface horizontal flow wetland is provided in Figure 15.

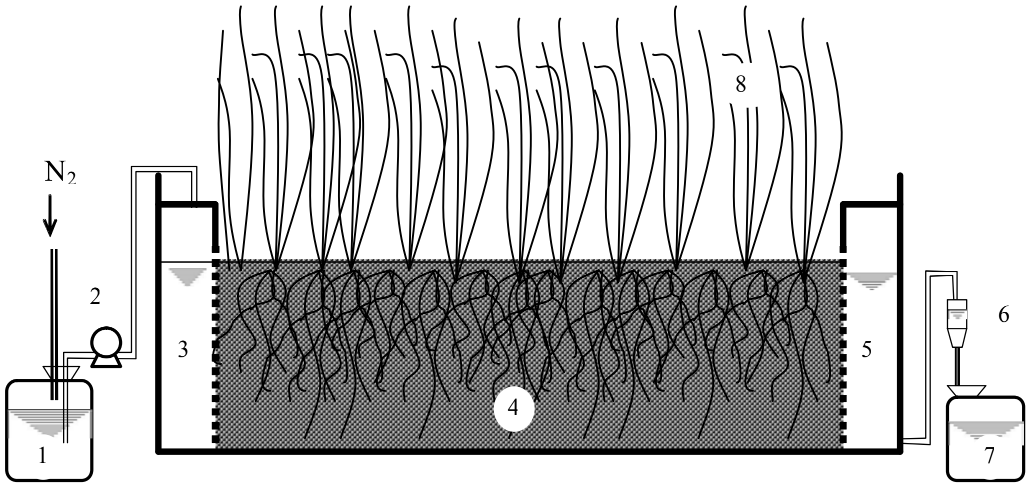

Figure 15 Horizontal flow laboratory-scale constructed wetland. (1 Feeding storage tank, 2 Pump, 3 Distribution chamber, 4 Gravel bed, 5 Outflow, 6 Flow meter, 7 Outflow storage tank, 8 Plants).

The wetlands consisted of a plastic container of 100 cm in length, 15 cm in width and 35 cm in height. The container was filled with 65.7 kg gravel (2-8 mm) up to a height of 30 cm and had a free pore water volume of 15.2 L; the water level was adjusted to 5 cm below the surface of the gravel bed. Sieves of perforated stainless steel were placed 3 cm in front of the inflow and outflow of the gravel bed. This free liquid volume should ensure an equal distribution of the inflow and a laminar liquid flow through the gravel bed (see Figure 15).

The soil material in the wetlands was washed gravel in a range between 2-8 mm. Wetland 2 (W2) and 3 (W3) were planted with macrophytes (*Juncus effusus*) with a density of 1573 and 1433 shoots m^{-2}, respectively. As a control no plants were grown on Wetland 1 (W1). Table 10 gives the main constructive details of the treatment units.

Table 10 Physical and operational characteristics of the laboratory-scale experimental wetlands

Characteristics of the wetlands systems	W_1 , W_2 and W_3
Constructed height, m	0.35
Height of the gravel bed, m	0.30
Height of the water level, m	0.25
Length, m	1.00
Width, m	0.15
Hydraulic retention time, d	5 – 2.5
Hydraulic loading rate, m d^{-1}	0.02 – 0.04
Flow rate, L d^{-1}	3.1 – 6.2

Each laboratory-scale subsurface horizontal wetland was fed separately from the same storage tank (30 L capacity). The storage tank was used for storing the synthetic wastewater (see 3.2.1) to be treated in the reactor. The tank had to be refilled before it became empty to ensure uninterrupted flow of wastewater. The storage tank with the synthetic wastewater (see 3.2.1) was kept anaerobic by bubbling nitrogen gas continuously through the head space of the storage tank.

3.3.3 Experimental conditions

The wetlands W1, W2 and W3 were run under three different experimental conditions (phases A, B and C) realised by different sulphide and sulphate concentrations in the feeding tank respectively by different hydraulic loading rates (see Table 11).

Despite the nitrogen atmosphere in the feeding tank the sulphide concentration was not stable; some thiosulphate existed already as an impurity of the sodium sulphide respectively was formed from sulphide by autoxidation during storage. That is why the artificial wastewater was prepared every 3 days anew.

Table 11 Operation conditions (phases A, B and C) of the experimental wetlands W1, W2 and W3 realised by different sulphide and sulphate concentrations respectively by different hydraulic retention times of the artificial wastewater.

Parameter	Phase		
	A	B	C
Hydraulic retention time, d	5	2.5	2.5
Sulphide concentration, mg S L^{-1}	5	5	15
Sulphate concentration, mg S L^{-1}	1.7	$1.7^{1)}$ / $8.2^{2)}$	8.2
Hydraulic loading rate, m d^{-1}	0.02	0.04	0.04
Organic loading rate, g BOD_5 m^{-2} d^{-1}	1.6	2.9	3.0

$^{1)}$ W1 (unplanted) and W2 (planted), $^{2)}$ W3 (planted)

The experimental wetlands were placed in the phytotechnical laboratory (see Figure 16) operating under defined environmental conditions with a temperature of 16-22°C simulating an average summer day in moderate climates (Wiessner et al., 2005a). The operation periods run from middle Octobre 2005 to September 2006.

Figure 16 Experiment set up of the laboratory scale subsurface horizontal wetlands: W1 (unplanted), W2 and W3 (planted).

3.3.4 Sampling

Water samples were taken weekly from the middle of the inlet, middle and outlet of wetland unit with a syringe (60 ml) and a long needle which was rinsed in advance with N_2 gas to minimise autoxidation of sample ingredients.

3.4 Analytical methods

3.4.1 Dissolved sulphide

The concentration of free sulphide was determined with an ion-specific Ag^+/S^{2-} electrode (Silver/Sulphide-Electrode Ag 500, WTW, Germany) in a 6 ml sub-sample fixed immediately after collection with sulphide antioxidant buffer containing sodium hydroxide, sodium EDTA, and ascorbic acid according the WTW's instruction. The detection limit of sulphide was 0.003 mg S^{2-} L^{-1}.

3.4.2 Sulphite and thiosulphate

The inorganic highly reactive sulphur compounds sulphite and thiosulphate in the water samples were analyzed by high performance liquid chromatography (HPLC, modified method according to Rethmeier et al., 1997). The sulphur components were derivatized by monobromobimane to yield fluorescent derivatives. The derivatized sulphur compounds were detected by fluorescence emission at 480 nm. The HPLC (Beckman, USA) was equipped with a 250 mm*4 mm column filled with LiChrosphere® 60 RP select B (5 µm, Merck, Germany) and a fluorescence detector (Shimadzu, Japan). The eluents were 0.25 % acetic acid, pH 4 (solvent A) and 100 % methanol (solvent B). The flow rate of the eluent was 1 ml min^{-1} and the gradient was programmed as follows:

0-5 min	88 % A, 12 % B isocratic
5-13 min	12-30 % B linear gradient

13-16 min	30 % B isocratic
16-34 min	30-60 % B linear gradient
34-36 min	60-100 % B linear gradient
36-39 min	100 % B isocratic
39-39.1 min	100-12 % B linear gradient
39.1-42 min	88 % A, 12 % B isocratic

The lowest detectable concentration was 0.08 mg L^{-1} for sulphite and 0.112 mg L^{-1} for thiosulphate.

3.4.3 Elemental sulphur

Elemental sulphur was also determined according Rethmeier et al., 1997 by extracting samples with chloroform and the subsequent detection by HPLC (Beckman, USA) using a Li Chrospher 100, RP 18 column (5 µm, Merck, Germany) and equipped with a UV-detector at 263 nm. The detection limit for elemental sulphur was about 0.064 mg L^{-1}.

3.4.4 Total carbon and total organic carbon

The total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) of the inflow and outflow of the reactors were analyzed using TOC analyzers (Shimadzu, TOC 600, Duisburg, Germany).

3.4.5 Ion chromatography analysis (IC)

Concentration of ammonia, nitrite, nitrate and sulphate were analyzed by ion chromatography (DIONEX 100, columns AG4A-SC/AS4A-SC (for anions) and

CG12A/CS12A (for cations); Idstein, Germany) using a UV detector for nitrite and nitrate at a wave length of 215 nm and a conductivity detector for the other ions. The self generating suppressor ASRS-Ultra 4mm (for anions) and CBES-I 4 mm (for cations) were used (Wießner et al., 2005a).

3.5 Other parameters

3.5.1 Redox potential (Eh) and pH measurement

The redox potential in the Planted Fixed Bed Reactor was measured by the Pt4805-S7/120 combination Redox, METTLER TOLEDO, and the pH by the pH-electrode Sentix 41. Both parameter were measured on-line and recorded by a microprocessor Standard (pH-ION-Meter pMX 3000/pH, WTW) which allows the measurements on-line every 20 minutes.

In the laboratory-scale horizontal subsurface wetland redox potential was measured in situ every 5 minutes during 10 minutes.

The proper functioning of the electrodes are tested with WTW solution for redox potential (Pt/Ag/AgCl in 1 M KCl, +220 mV/25 °C) and for the pH, two different pH buffers (pH 4.01 and pH 7.00) solutions were used. Redox potential values were converted to the potential relative to the normal hydrogen reference electrode (Eh) taking the sample temperature into account.

3.5.2 Evapotranspiration

Initial and final conditions (weight and volume) feeding (3 to 4 days) were measured in order to determine the evapotranspiration of the system. The reactor design ensures that evaporation can be neglected. The transpiration by the plants was controlled by balancing the inflow and outflow amounts of water. The total amount of water loss was divided by the time and the area to calculate the specific water loss (transpiration rate, L $m^{-2}d^{-1}$).

3.5.3 Shoot density

The number of the plants was estimated periodically, at approximately 30 days intervals during all experiments by counting the number of total (green and yellow) shoots and divided by the area to calculate the density of the plants.

3.5.4 Gravel analysis

The gravel bed used in this experiment was previously washed with tap water to remove unattached small particles before processing and then heated in a drying oven set at 105 °C for 2 hours. For two gravel samples were determined the gravel size, the density, porosity and uniformity coefficient.

The granulometric distribution of sizes of the gravel bed was made with the Vibratory Sieve Shaker Analysette 3 (FRITSCH). The density of the gravel was measured based on the water replacement method proposed by Balck (1986) and ASTM (1994). The porosity was calculated, dividing the volume of water that could be poured, in each graduate glass, by the total volume of the material. Uniformity coefficient (Cu) is defined like the ratio between material accumulated between the 60 and 10 percent in the granulometric curve. Table 12 shows a summary of the results.

Table 12 Characteristic of the size, density (ρ), porosity (P) and uniformity coefficient (Cu) used gravel in the Planted Fixed Bed Reactors-PFBR.

Sample	Size (mm)	ρ (kg m^{-3})	P (%)	$Cu = D_{60}/D_{10}$
1	2-8	1482	42.8	1.07
2	2-8	1543	40.6	1.12
Average	2-8	1512	41.7	1.10

3.5.5 Total sulphur

Total sulphur was defined as the sum of sulphide, sulphate, thiosulphate, sulphite and elemental sulphur.

3.5.6 Total nitrogen

Total nitrogen was defined as the sum of ammonia, nitrite and nitrate. Organic nitrogen was assumed to be negligible.

3.5.7 Specific removal rate

The specific removal rates of the wetland systems were calculated as the difference between the specific inflow and outflow loads (mg m^{-2} d^{-1}).

Specific (inflow/outflow*) rate = [concentration (mg L^{-1}) x flow rate (L d^{-1})]/area (m^2)

* The outflow rate include the loss water of evapotranspiration

3.5.8 Data analysis

The range and variation in physicochemical parameter in PFBR's and in planted and unplanted laboratory scale wetlands has been summarized by Principal Components Analysis (PCA) where the multivariate data has been reduced to two dimensions and displayed as "bi-plots" (Statistical Sciences Inc. 1993). PCA allows studying the relationship among descriptors and object in order to summarize important data sets and facilitate the interpretation of the data. PCA characterizes the main trends of variation of the objects (e.g. sampling sites) with respect to all descriptors (e.g. physicochemical parameters). A scatter of the objects is represented in a multidimensional diagram, with as many axes as there are descriptors in the study. The analysis summarizes the range of variation of a multivariate data set by reducing it to two dimension and display as bi-plots.

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.

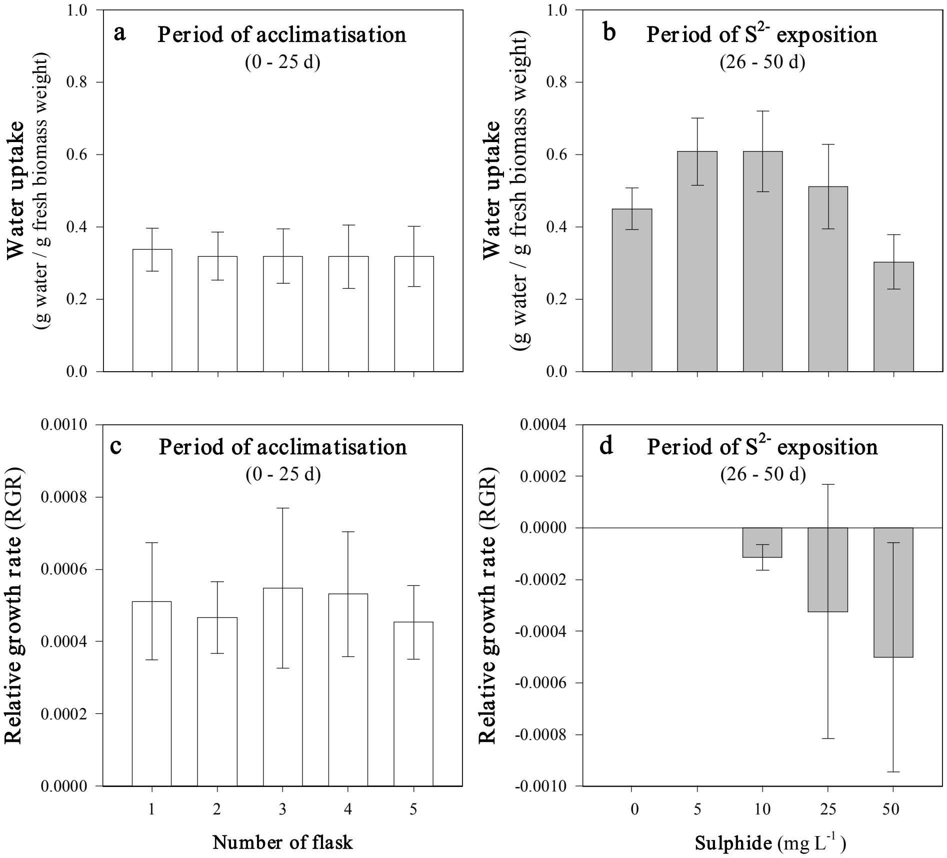

Figure 17 Mean specific water uptake (a and b) relative growth rate (c and d) of *J. effusus* depending on the exposition of sulphide in the nutrient solution. Bars shown with standard error of the mean; n = 10 (a, c) and 16 (b, d) respectively.

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

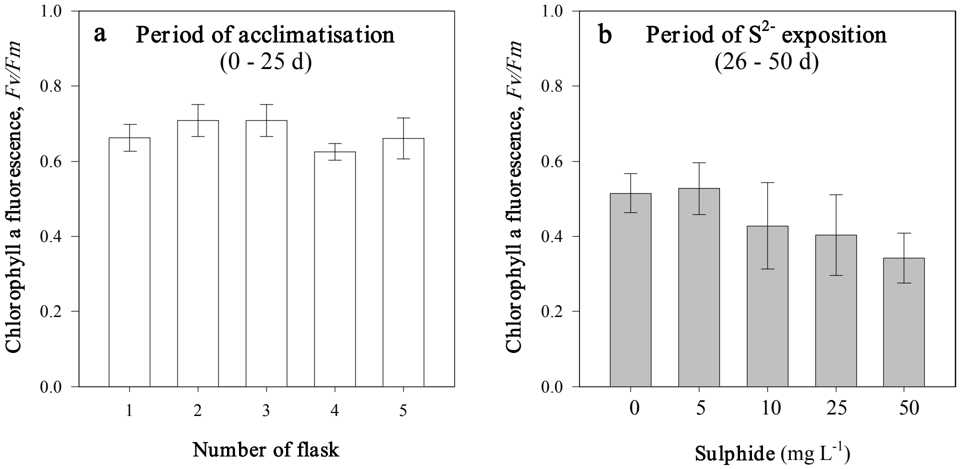

Figure 18 Chlorophyll *a* fluorescence of *J. effusus* depending on the exposition of sulphide in the nutrient solution (bars are shown with standard error of the mean; n = 10 a) and 12 b) respectively.

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.

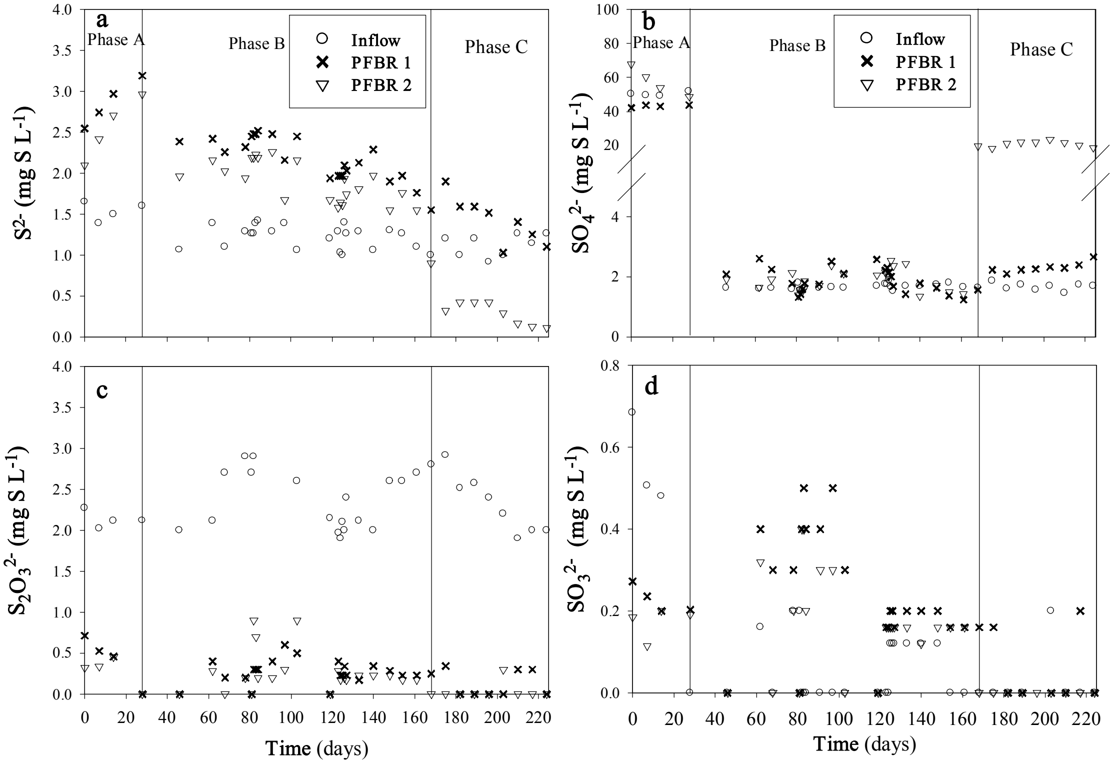

Figure 19 Sulphide a), sulphate b), thiosulphate c) and sulphite d) concentration of the inflow and outflow of the Planted Fixed Bed Reactors

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.

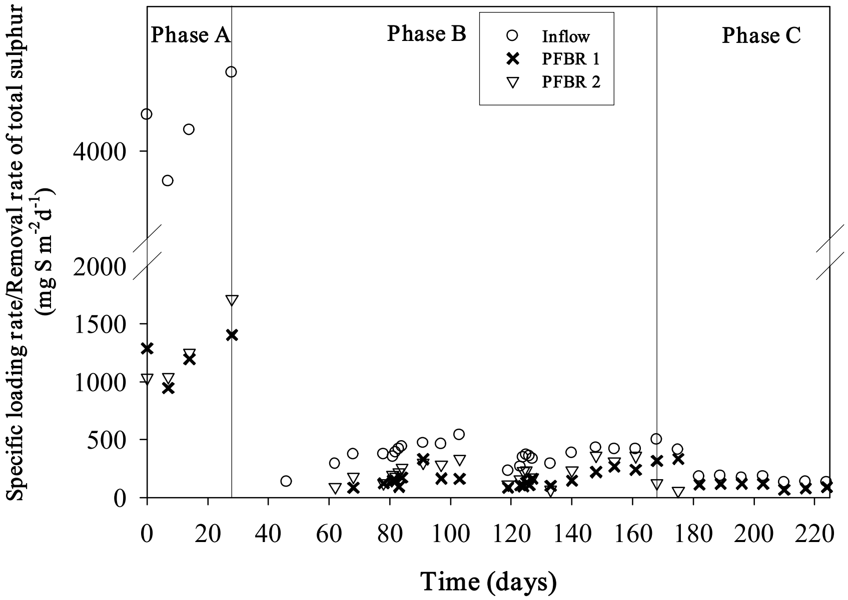

Figure 20 Specific loading and removal rate of total sulphur of the Planted Fixed Bed Reactor.

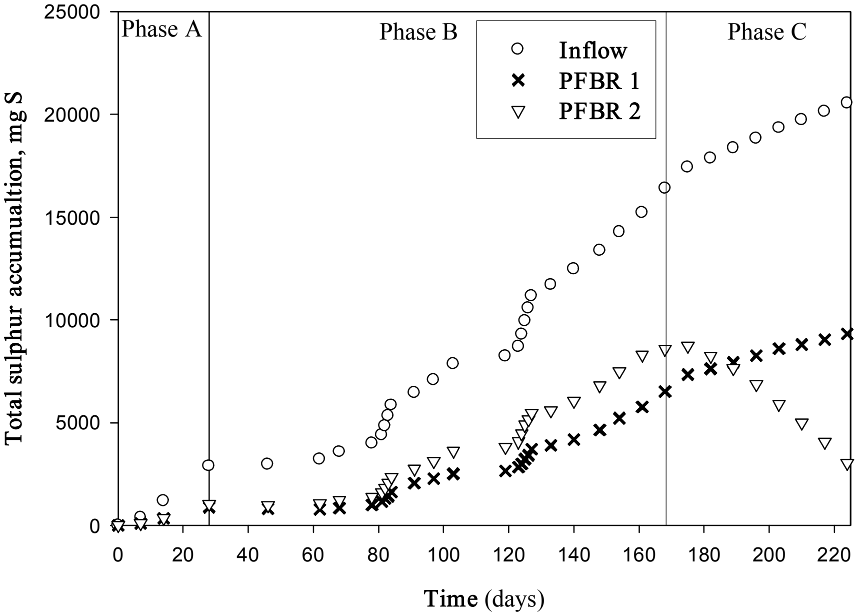

Figure 21.Total sulphur accumulation of the both Planted Fixed Bed Reactors

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.

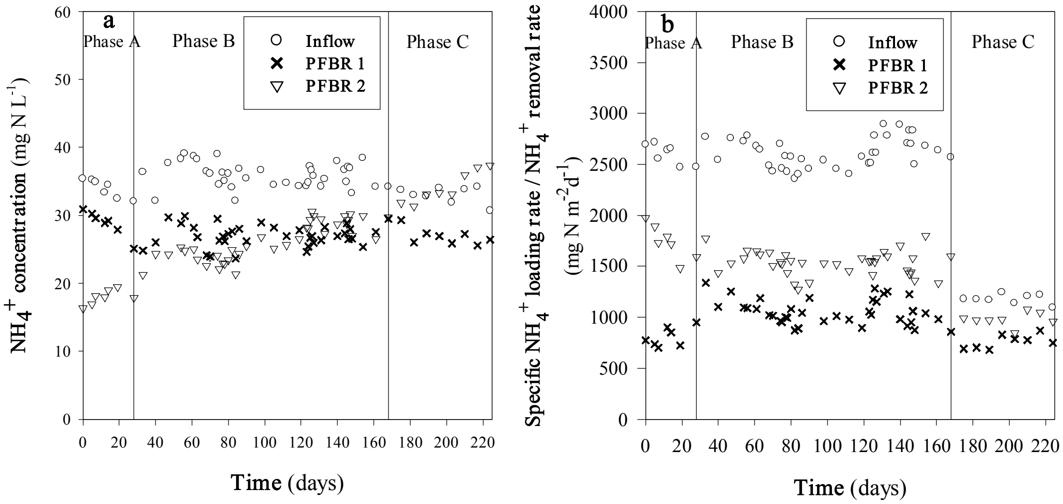

Figure 22 Ammonia concentration a) and specific loading and ammonia removal rate b) of the Planted Fixed Bed Reactor.

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.

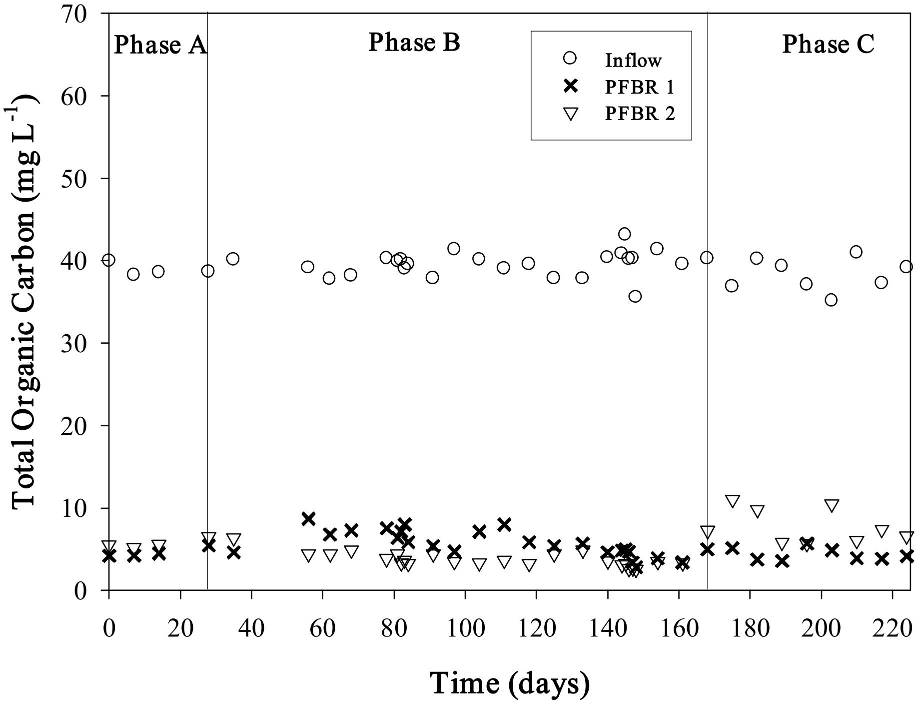

Figure 23 Total organic carbon concentrations of the Planted Fixed Bed Reactors

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.

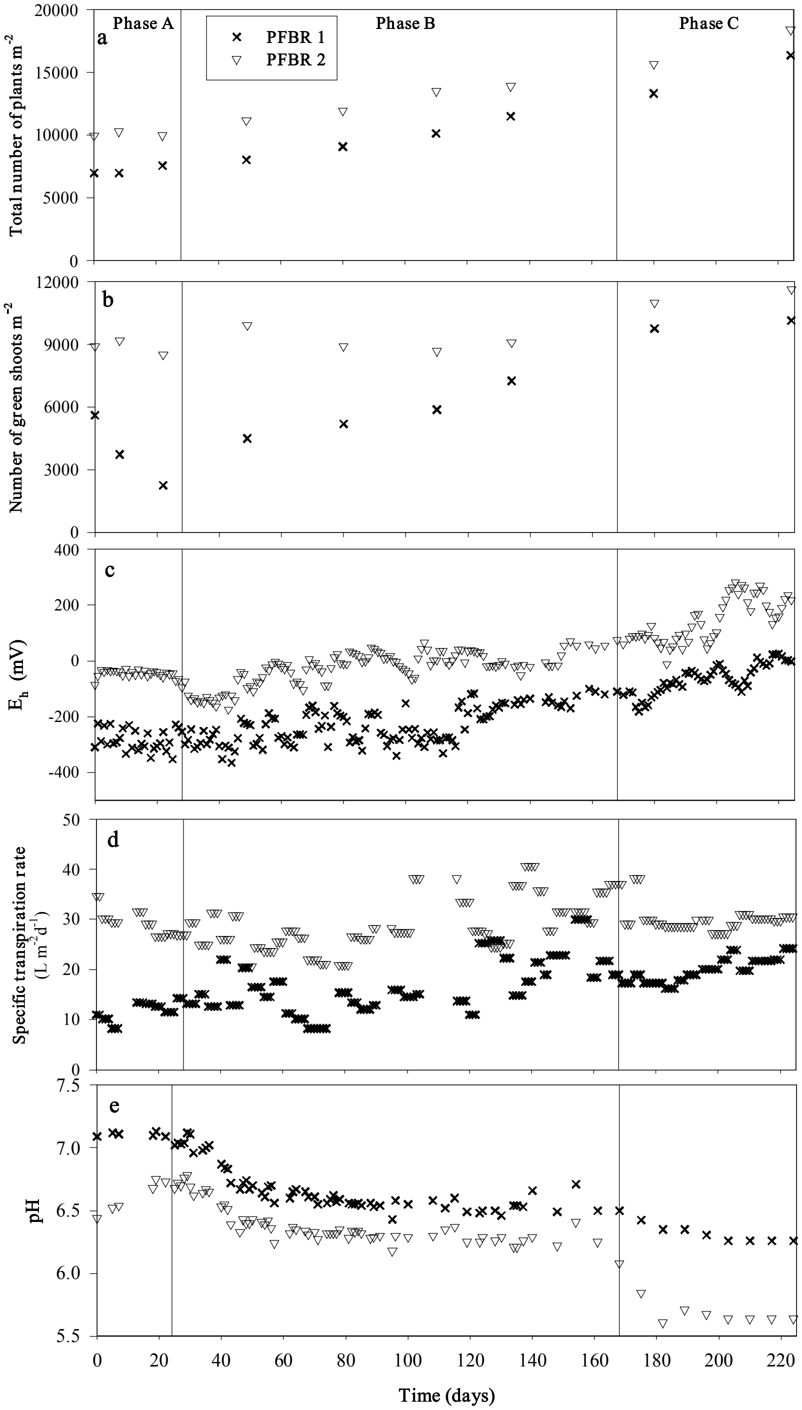

Figure 24 Number of total shoots a), green shoots b), redox potential c), specific transpiration rate d) and pH e) of the both Planted Fixed Bed Reactors.

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.

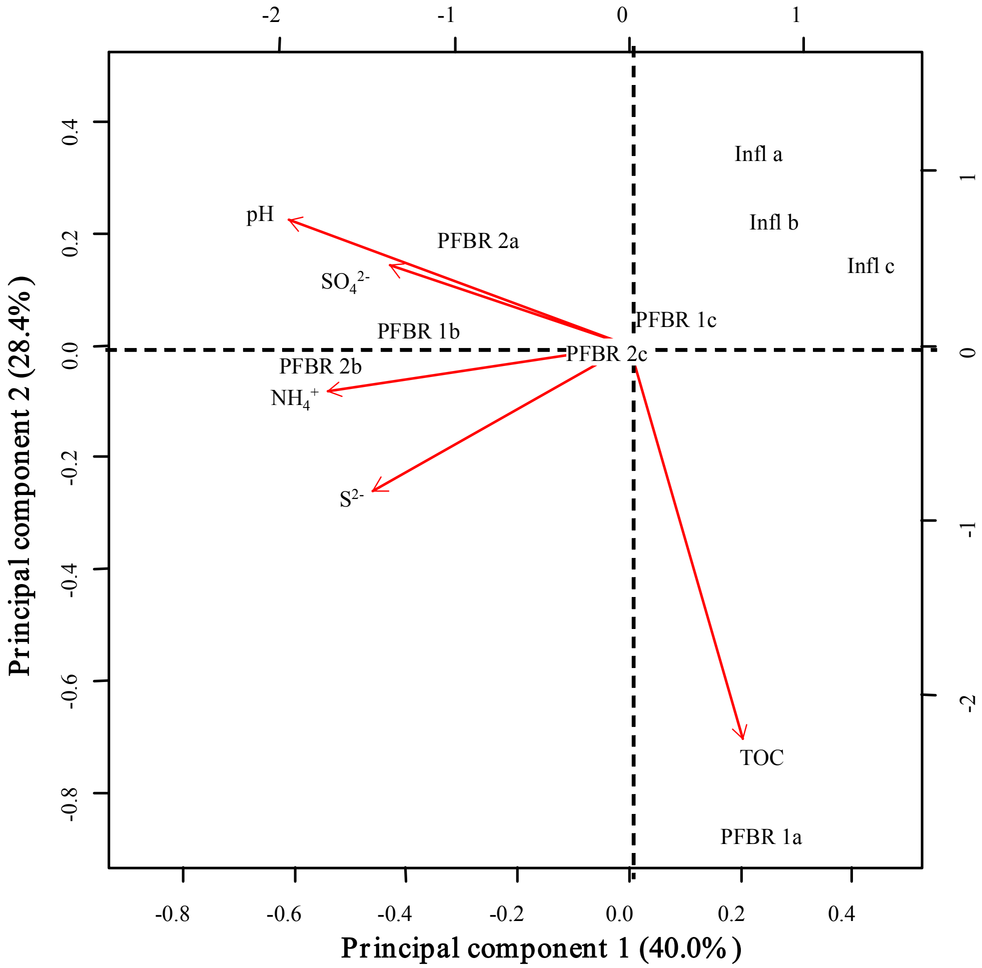

Figure 25 PCA ordination plot of water samples according to sulphur species, ammonia and TOC in the Planted Fixed Bed Reactors during experimental phases A, B and C. A code identifies each bed and phase: the letters refers to inflow (Infl) and Planted Fixed Bed Reactor (PFBR) name, followed by number 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).

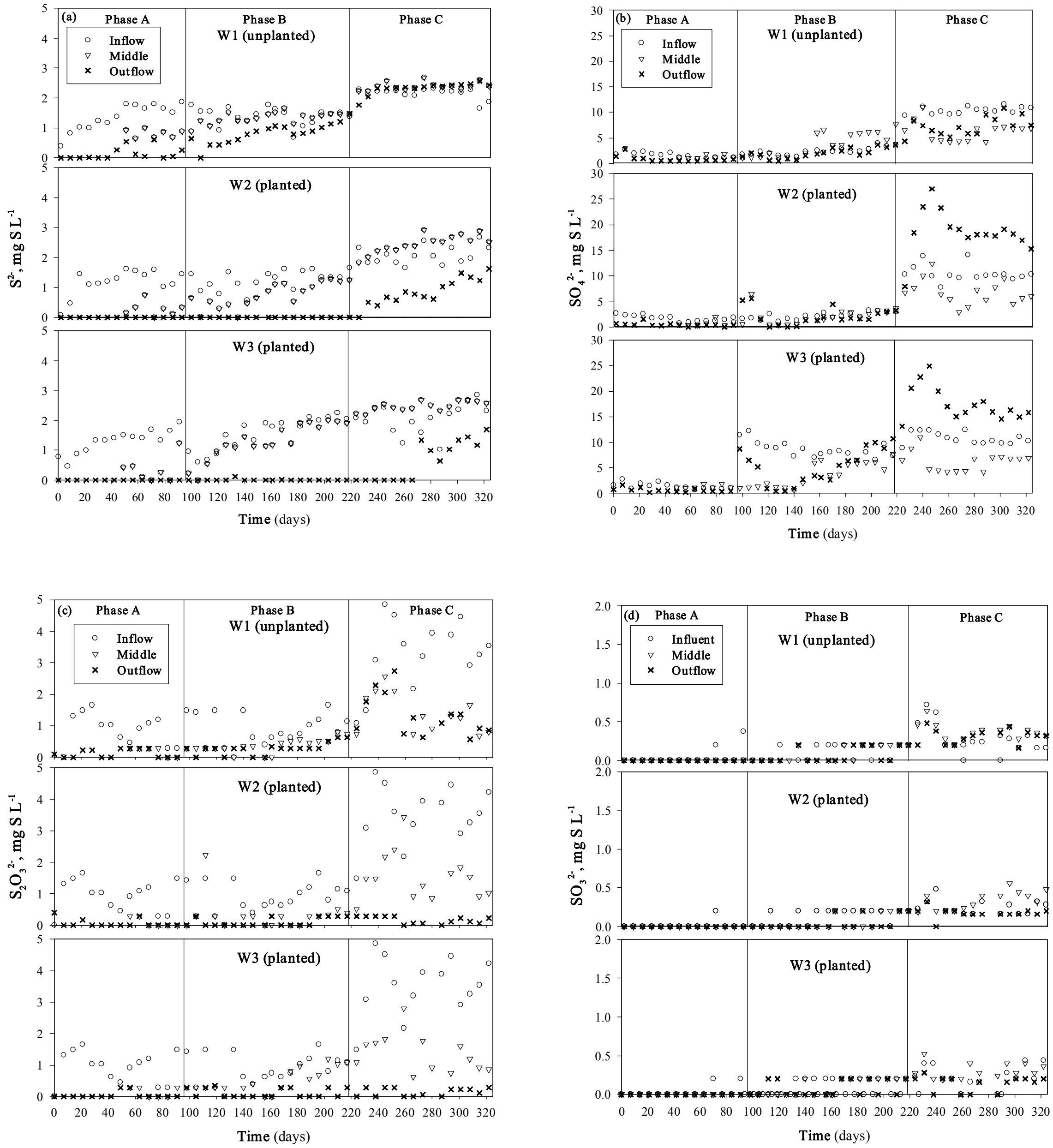

Figure 26 Sulphur concentrations of the inflow, middle and outflow of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

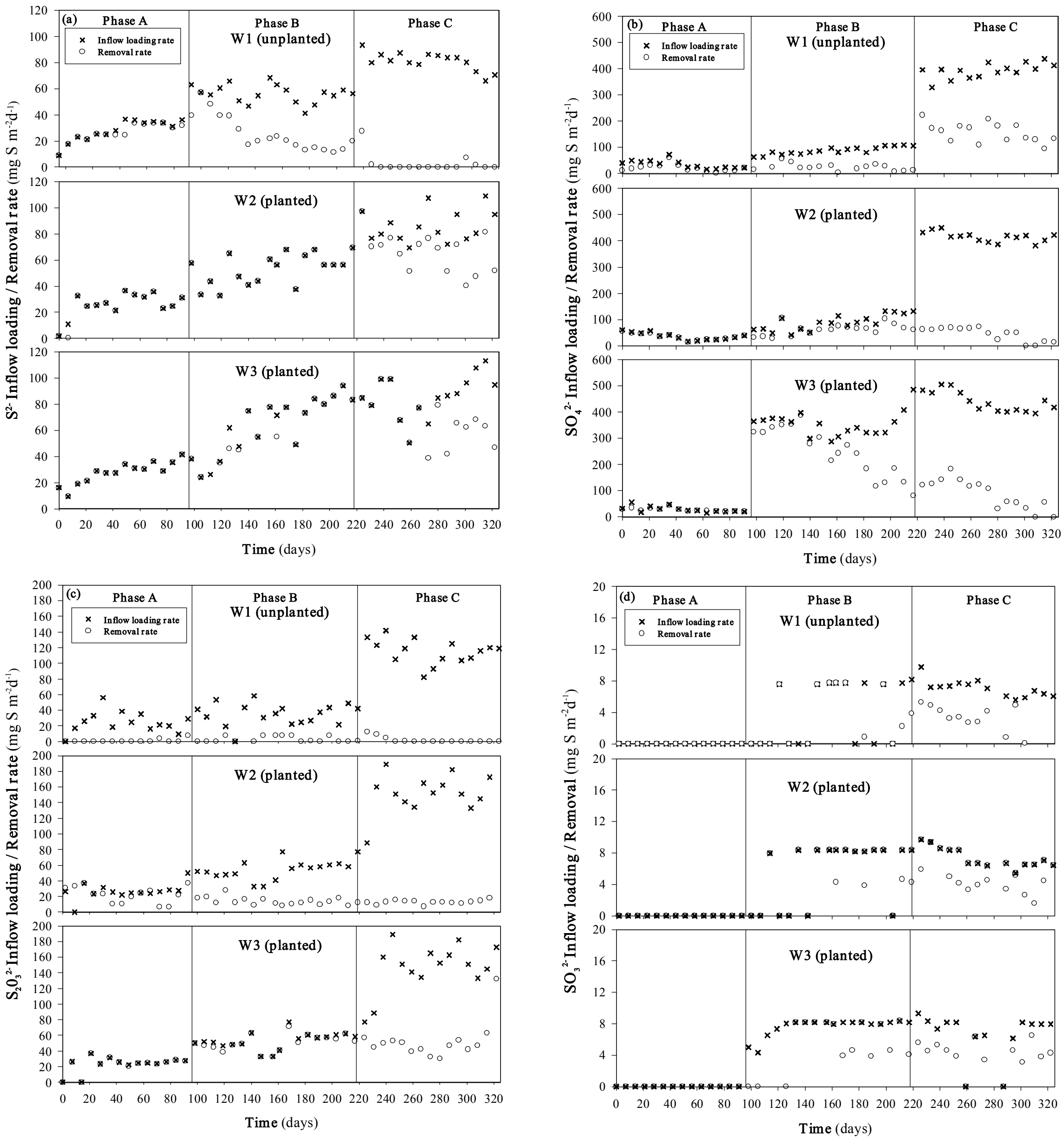

Figure 27 Specific loading rate and removal rate of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

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.

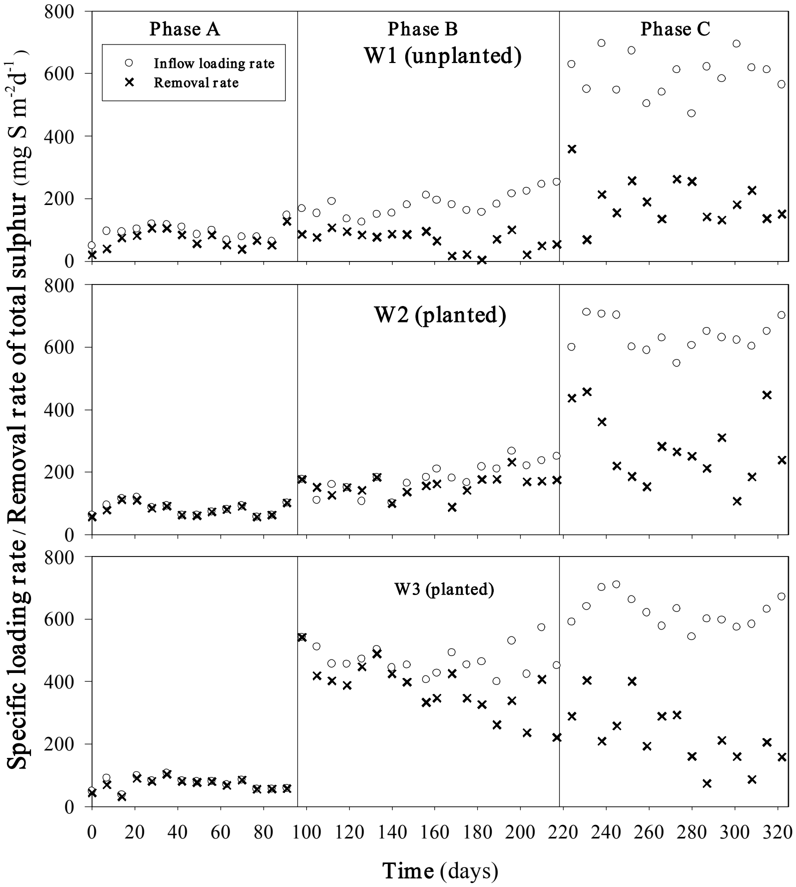

Figure 28 Specific loading rate and removal rate of total sulphur of the subsurface horizontal laboratory-scale constructed wetlands.

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.

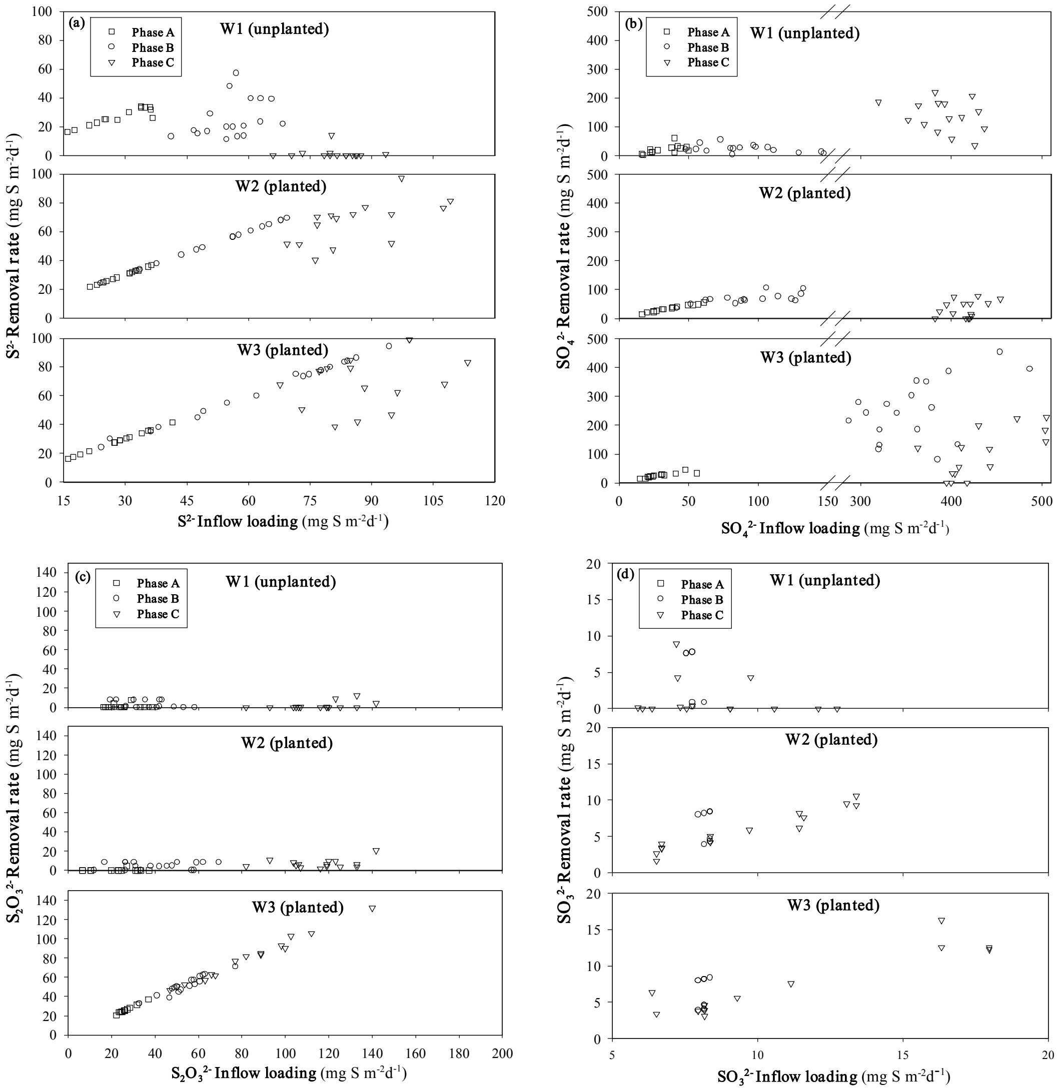

Figure 29 Correlations between inflow loading and removal rate of sulphide a), sulphate b), thiosulphate c) and sulphite d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

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.

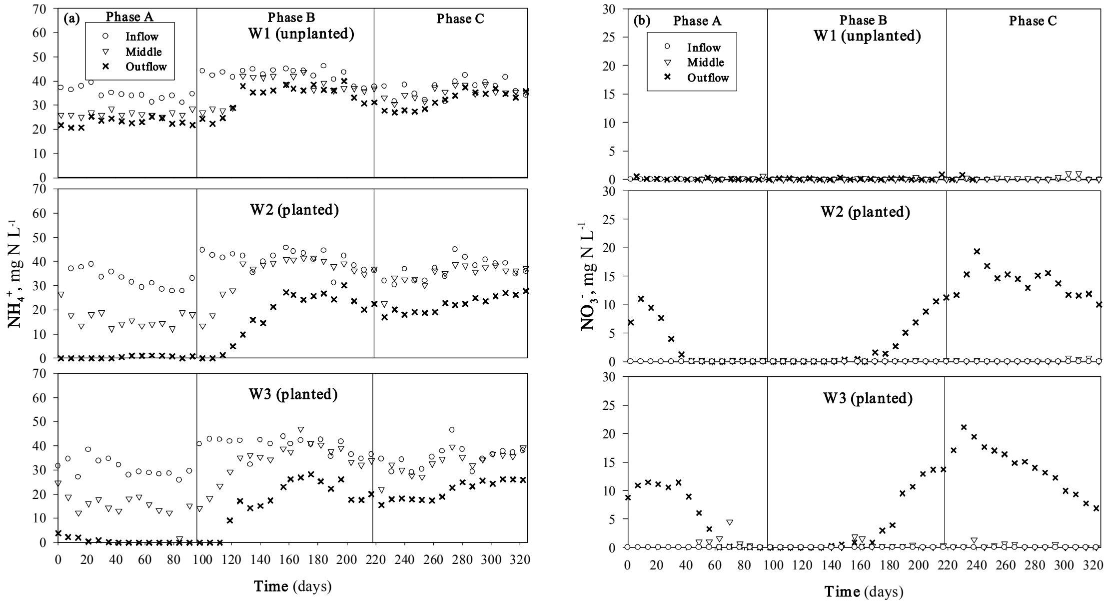

Figure 30 Ammonia a) and nitrate b) concentration of the inflow, middle and outflow in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

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

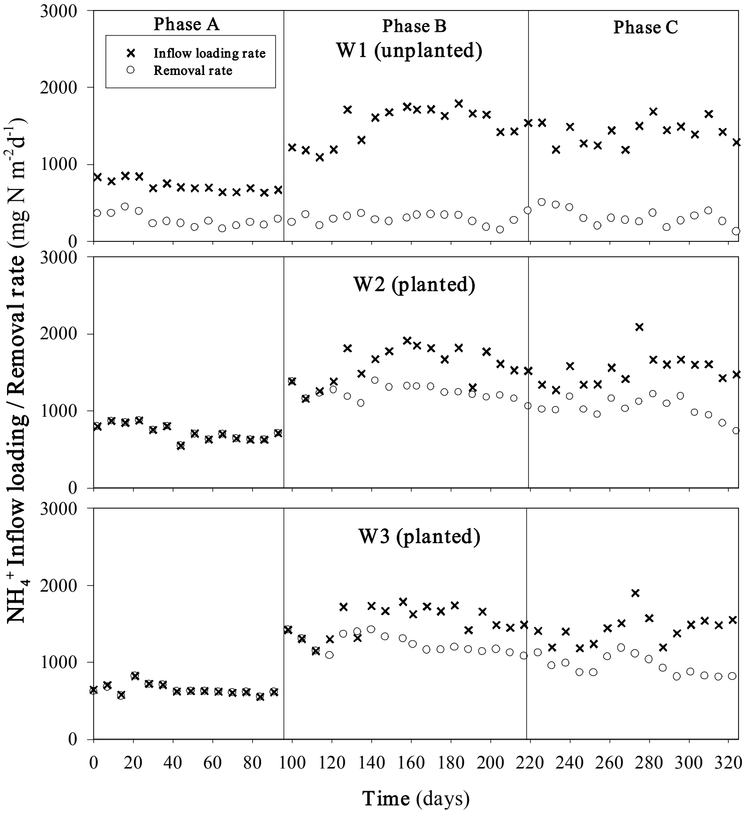

Figure 31 Specific loading rate and removal rate of ammonia of the subsurface horizontal laboratory-scale constructed wetlands.

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.

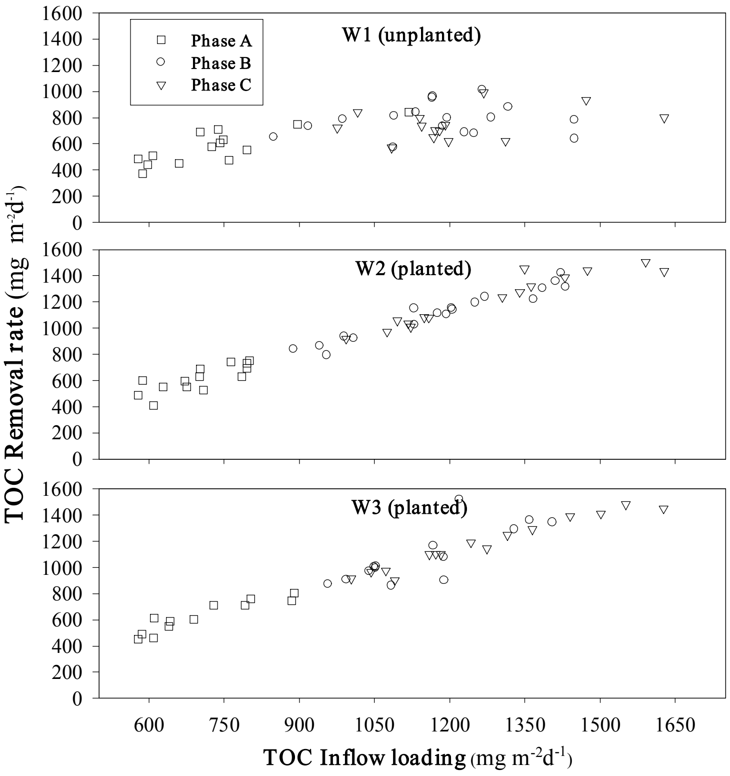

Figure 32 Correlations between the area specific inflow loading and removal rate of total organic carbon-TOC in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

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.

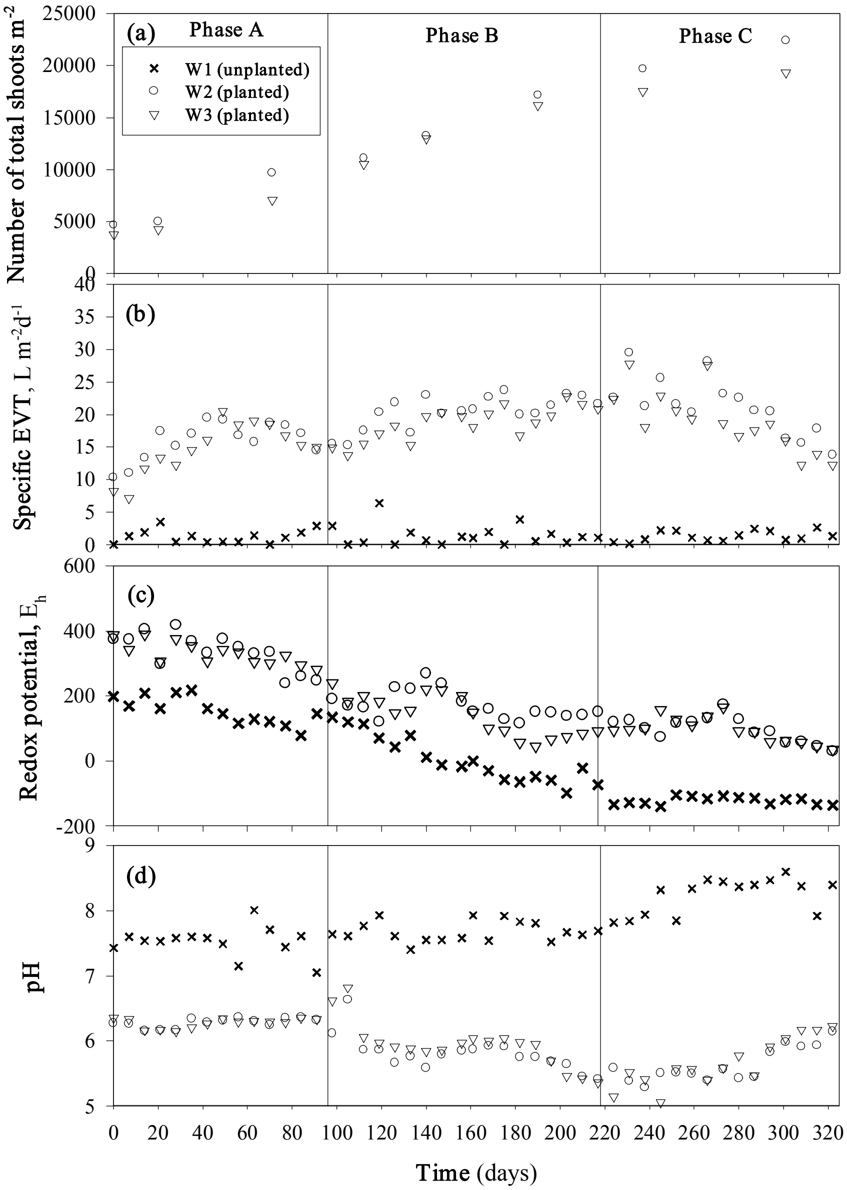

Figure 33 Number of total shoots a), specific evapotranspiration b), redox potential c) and pH d) in unplanted (W1) and planted (W1 and W2) subsurface horizontal laboratory-scale constructed wetlands.

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.

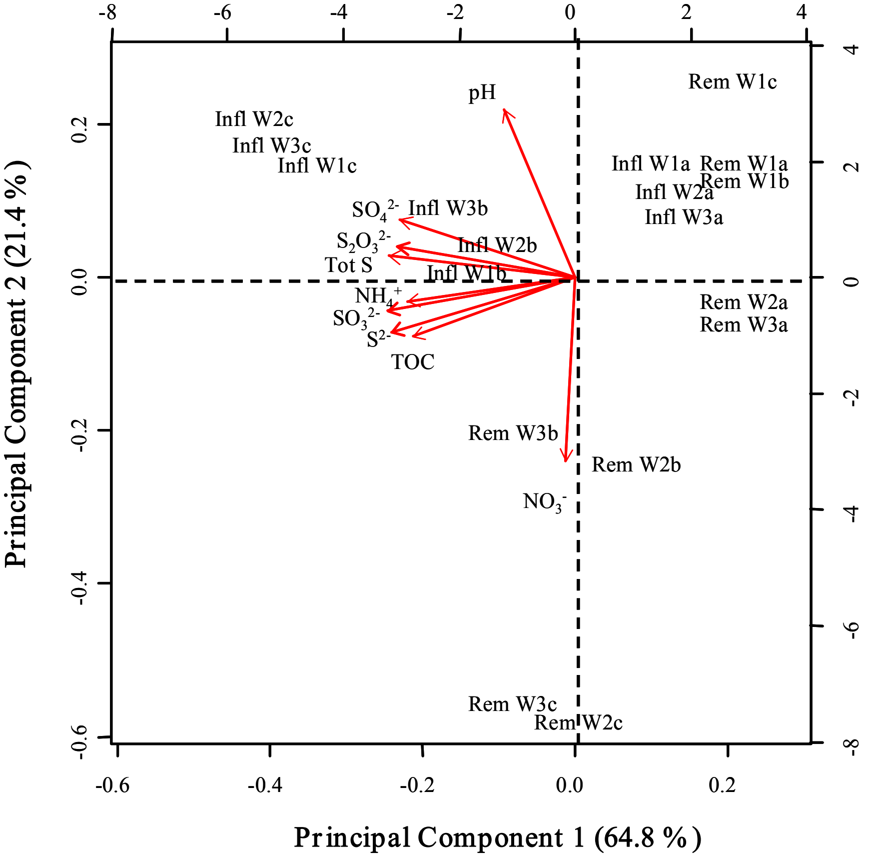

Figure 34 PCA ordination plot of water samples according to sulphur species, ammonia, nitrate and TOC in the unplanted control bed (W1) and planted beds (W2 and W3) during experimental phases A, B and C. A code identifies each bed and phase: the letters refers to inflow (Infl) and removal (Rem) name,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

5 Summary and Conclusions

Energy saving low-cost technologies for wastewater treatments are highly needed as well in developing as in industrialized countries. Especially the anaerobic methanogenic fermentation technology for wastewater offers a high energy saving potential – even a useful biogas is produced. So, already in many countries, especially in the tropics, domestic sewage treatment is often realised by an anaerobic fermentation step such as a septic tank, an anaerobic filter etc. By such a treatment step the biochemical oxygen demand (BOD) of the wastewater is considerably reduced, but it contains still relatively high residual BOD, ammonia and sulphide formed by the bacterial dissimilatory sulphate reduction. Because of this H_2S content and the poor effluent quality (relatively high BOD and ammonia) the anaerobic fermentation of wastewater needs a further post-treatment step.

Sulphide toxicity

Descriptions of sulphide toxicity were mainly done in stagnant waterlogged soils like paddy fields were the concentration gradients around the helophyte roots are more or less undisturbed. Contrary to these stagnant waterlogged soils in constructed wetlands the water with its ingredients is streaming through the “rooted soil filter”. By this, the oxygenated “protection” layer of the helophyte roots is permanently disturbed especially in case if the streaming water contains highly toxic H_2S. That is why, toxicity tests should be executed under conditions were the roots are directly exposed to the actual toxicant concentration like in hydroponic culture.

In this study, the physiological responses of *J. effusus* according to the effects of different concentrations of sulphide on its growth and constitution, was investigated under controlled laboratory hydroponic conditions. Concentration-limits for all the evaluated parameters i.e. relative growth rate, water uptake, and chlorophyll fluorescence were defined for this species. Such laboratory experiments are necessary to optimise the design and operation of constructed wetlands for the treatment of H_2S containing waters. Unfortunately, no comparable data are available for other wetland species under similar conditions.

The most striking result is that there are no big differences between the sensitivity of relative growth rate, the efficiency of photosynthesis and water uptake in *J. effusus*. These parameters were found not to be influenced by concentrations of sulphide lower than 10 mg L^{-1}. By this, *J. effusus* is well suitable for the application in constructed wetlands to treat sulphide-loaded wastewater as effluents from anaerobic digestion of domestic sewage.

Post-treatment model experiments in a laboratory-scale macro-gradient-free wetland system (*Planted fixed Bed Reactor*)

Previous methods for the investigation of rhizospheric processes are limited in their use for further development in application of treatment wetlands. The usual inflow/outflow characterization of more or less large systems with internal gradients has to be supplemented by small-scale gradient-free model investigations. This methodical requirement has been addressed by the "Planted Fixed-Bed Reactor". The principle of the reactor ensures macro-gradient-free conditions for investigating redox processes inside the rhizosphere. Using *Juncus effusus* plants, gravel as soil material and a hydraulic retention time of 5 and 10 days, long-term investigations were carried out treating synthetic anaerobic reactor effluent of domestic sewage. Long-term (over month) effects on removal of contaminants and the variability of the redox conditions were monitored.

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

Treatment of artificial sulphide containing wastewater in planted and unplanted subsurface horizontal flow laboratory-scale constructed wetlands

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. Furthermore the results 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.

The plants affected a clear stimulation of the sulphide and ammonia removal rates. Sulphide concentration in the range of 1.5 - 2.0 mg L^{-1} were tolerated by the plants and completely removed in the planted model wetlands; sulphide concentration of > 2.0 mg L^{-1} caused instabilities in sulphide and nitrogen removal. Area specific sulphide removal rates of up to 94 mg sulphide $m^{-2}d^{-1}$ were achieved in the planted beds at hydraulic retention times of 2.5 d. Sulphate affected the sulphide removal. While in the unplanted control bed an almost stable removal in the range of 150 - 300 mg N $m^{-2}d^{-1}$ was observed variations of hydraulic retention time, sulphide and sulphate concentrations influenced the ammonia removal rate within the planted beds in a broader range (600 - 1,400 mg N $m^{-2}d^{-1}$).

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.

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

Concluding remarks

The results showed that sulphide oxidation, nitrification, denitrification and sulphate reduction occur simultaneously in the rhizosphere of treatment wetlands caused by dynamic redox gradients (aerobic-anaerobic) conditions. 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, changes of micro-environmental conditions in the rhizosphere due to redox potentials and sulphur deposits, nutrient mobilization or immobilization, and biofilm formation.

In general, the results of the experiments substantiate the suitability of post-treatment of anaerobic reactor effluents in subsurface horizontal flow constructed wetlands.

In comparison to ponds and surface-flow wetlands this type of wetland ensures considerable lower toxic and smell intense H_2S emmision into the atmosphere.

In the laboratory-scale system high treatment efficiency concerning sulphide removal was shown. The model system was relative shallow (25 cm) in comparison to full-scale systems. Nevertheless, there is to expect that a deeper bed will not improve the treatment efficiency concerning sulphide removal. The deeper zones with less root density function more or less only as an "anaerobic filter".

In general sulphide inflow concentration should be controlled on a regular basis and should not exceed 5 mg L^{-1}; if necesary, by wetland effluent recirculation for diluting the sulphide inflow concentration this crucial parameter can be guaranteed.

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7 Appendix

Appendix A: Abbreviation directory

Appendix B: List of chemical compounds

Appendix C: Odorants associated with sewage treatment works (Abbott, 1993; Bonnin et al., 1990; Brennan, 1993; Cheremisinoff, 1988; Koe, 1989; Vincent and Hobson, 1998; Young, 1984; Metcalf and Eddy, 1995)

Appendix D: Detectable H_2S concentration and physiological response

Appendix E: Changes of the redox potential in the rhizosphere of the Planted Fixed Bed Reactor at the day 200 (experimental phase C).

Appendix A: Abbreviation directory

AMD	acid main drainage
Anammox	anaerobic ammonia oxidation
APS	adenosine phosphosulphate
ASTM	american society for testing and material
AVS	acid volatile sulphide
BOD_5	5-day biochemical oxygen demand
d	day
DIN	german institute for standardization
DO	dissolved oxygen
DOC	chemical oxygen demand
Eh	redox potential
EPS	environmental protection and safety
et al.	and others, (Latin: et alteri)
Fig.	figure
FSW	free surface wetland
*F*o	fluorescence of photosystem II
Fm	fluorescence following a pulse of saturation light
Fv/*Fm*	photochemical efficiency of photosystem II
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
IC	ion chromatography
J. effusus	*Juncus effusus* sp.
PAM 2000	pulse amplitude modulated fluorometer
PFBR	planted fixed bed reactor
ppm	part per million
ppmv	parts per million by volume
ppmb	parts per billion by volume
PCA	principal component analysis
PS II	photosystem II
RGR	relative growth rate
sp.	species, (Latin: species)
SRB	sulphate reducing bacteria
SSW	subsurface wetland
Tab.	table
TC	total carbon
TKN	total Kjeldahl nitrogen

TN	total nitrogen
TOC	total organic carbon
TSS	total suspended solid
UASB	up flow anaerobic sludge blanket
UFZ	Helmholtz Centre for Environmental Research
WW	wastewater
WWT	wastewater treatment
W1	horizontal flow laboratory-scale constructed wetland one
W2	horizontal flow laboratory-scale constructed wetland two
W3	horizontal flow laboratory-scale constructed wetland three

Appendix B: List of chemical compounds

Name	Producer
Sodium acetate	Merk
Ammoniumchlorid	Merk
Sodium chloride	Merk
Magnesium chloride hexahydrate	Merk
Sodium dihydrogen phosphate dihydrate extra pure	Merk
Calcium chloride dihydrate	Merk
Sodium sulfide hydrate	Fluka

Appendix C: Odorants associated with sewage treatment works (Abbott, 1993; Bonnin et al., 1990; Brennan, 1993; Cheremisinoff, 1988; Koe, 1989; Vincent and Hobson, 1998; Young, 1984; Metcalf and Eddy, 1995).

Class	Compound	Formula	Character
Sulphurous	Hydrogen sulphide	H_2S	Rotten eggs
	Dimethyl sulphide	$(CH_3)_2S$	Decayed vegetables, garlic
	Diethyl sulphide	$(C_2H_5)_2S$	Nauseating, ether
	Diphenyl sulphide	$(C_6H_5)_2S$	Unpleasant, burnt rubber
	Diallyl sulphide	$(CH_2CHCH_2)_2S$	Garlic
	Carbon disulphide	CS_2	Decayed vegetables
	Dimethyl disulphide	$(CH_3)_2S_2$	Putrification
	Methyl mercaptan	CH_3SH	Decayed cabbage, garlic
	Ethyl mercaptan	C_2H_5SH	Decayed cabbage
	Propyl mercaptan	C_3H_7SH	Unpleasant
	Butyl mercaptan	C_4H_9SH	Unpleasant
	tButyl mercaptan	$(CH_3)_3CSH$	Unpleasant
	Allyl mercaptan	CH_2CHCH_2SH	Garlic
	Crotyl mercaptan	$CH_3CHCHCH_2SH$	Skunk., rancid
	Benzyl mercaptan	$C_6H_5CH_2SH$	Unpleasant
	Thiocresol	$CH_3C_6H_4SH$	Skunk., rancid
	Thiophenol	C_6H_5SH	Putrid, nauseating, decay
	Sulphur dioxide	SO_2	Sharp, pungent, irritating
Nitrogenous	Ammonia	NH_3	Sharp, pungent
	Methylamine	CH_3NH_2	Fishy
	Dimethylamine	$(CH_3)_2NH$	Fishy
	Trimethylamine	$(CH_3)_3N$	Fishy, ammoniacal
	Ethylamine	$C_2H_5NH_2$	Ammoniacal
	Diethylamine	$(C_2H_5)_2NH_2$	
	Triethylamine	$(C_2H_5)_3N$	
	Diamines, i.e. Cadaverine	$NH_2(CH_2)_5NH_2$	Decomposing meat
	Pyridine	C_6H_5N	Disagreeable, ittitating
	Indole	C_8H_6NH	Faecal, nauseating
	Scatole or Skatole	C_9H_8NH	Faecal, nauseating
Acid	Acetic (ethanoic)	CH_3COOH	Vinegar
	Butyric (butanoic)	C_3H_7COOH	Rancid, sweaty
	Valeric (pentanoic)	C_4H_9COOH	Sweaty
Aldehydes and ketones	Formaldehyde	$HCHO$	Acrid, suffocating
	Acetaldehyde	CH_3CHO	Fruit, apple
	Butyraldehyde	C_3H_7CHO	Rancid, sweaty
	Isobutyraldehyde	$(CH_3)_2CHCHO$	Fruit
	Isovaleraldehyde	$(CH_3)_2CHCH_2CHO$	Fruit, apple
	Acetone	CH_3COCH_3	Fruit, sweet
	Butanone	$C_2H_5COCH_3$	Green, apple

Appendix D: Detectable H_2S concentration and physiological response

Detectable Concentration	Physiological response
10 ppm	Beginning eye irritation
50-100 ppm	Slight conjunctivitis and respiratory after 1 hour exposure
100 ppm	Coughing, eye irritation, loss of sense of smell after 2-15 minutes. After respiration, pain in the eyes and drowsiness after 15-30 minutes followed by throat irritation after 1 hour. Several hours exposure results in gradual increase in severity of these symptoms and death may occur within the next 48 hours.
200-300 ppm	Marked conjunctivitis and respiratory tract irritation after 1 hour of exposure.
500-700 ppm	Loss of consciousness and possibly death in 30 minutes to 1 hour.
700-1000 ppm	Rapid unconsciousness, cessation of respiration and death.
1000-2000 ppm	Unconsciousness at once, with early cessation of respiration and death in a few minutes. Death may occur even if individual is removed to fresh air at once.

Appendix E: Changes of the redox potential in the rhizosphere of the Planted Fixed Bed Reactor at the day 200 (experimental phase C).