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.

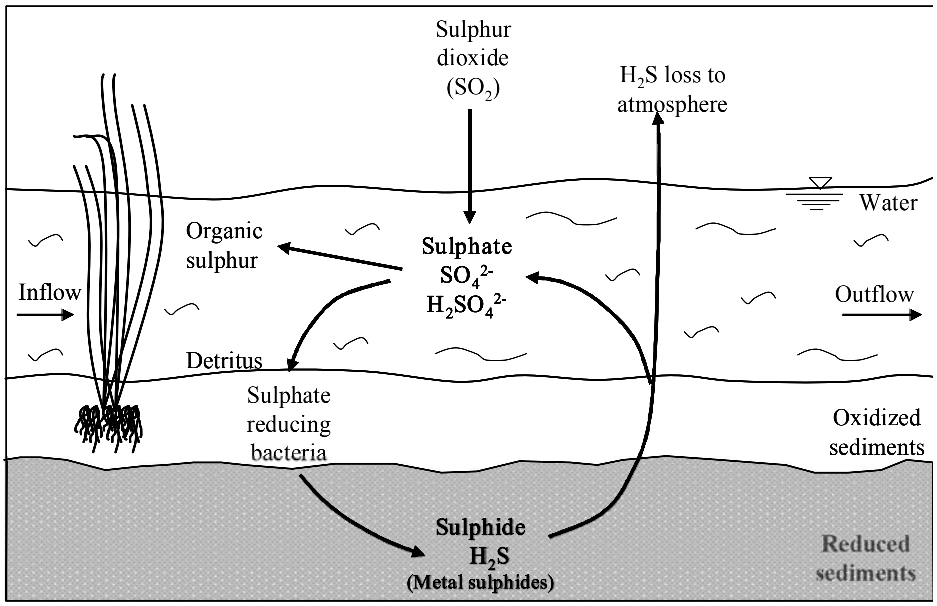

Figure 1 Typical wetland sulphur cycle.

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 ^oC, and as high as 110 ^oC in deep-sea hydrothermal vent sediments. At temperatures higher than 100 to 120 ^oC 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:

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

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

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:

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (2.7)$$

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

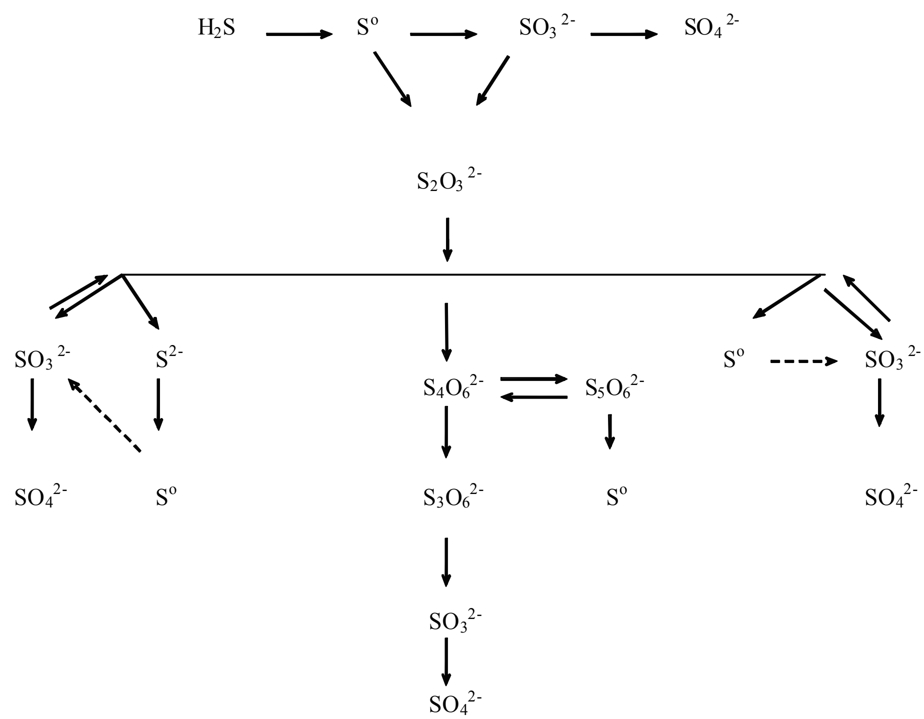

Figure 2 Possible oxidation pathways for reduced sulphur species to sulphate by *Thiobacillus* (Goldhaber and Kaplan, 1974).

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:

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

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

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

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.

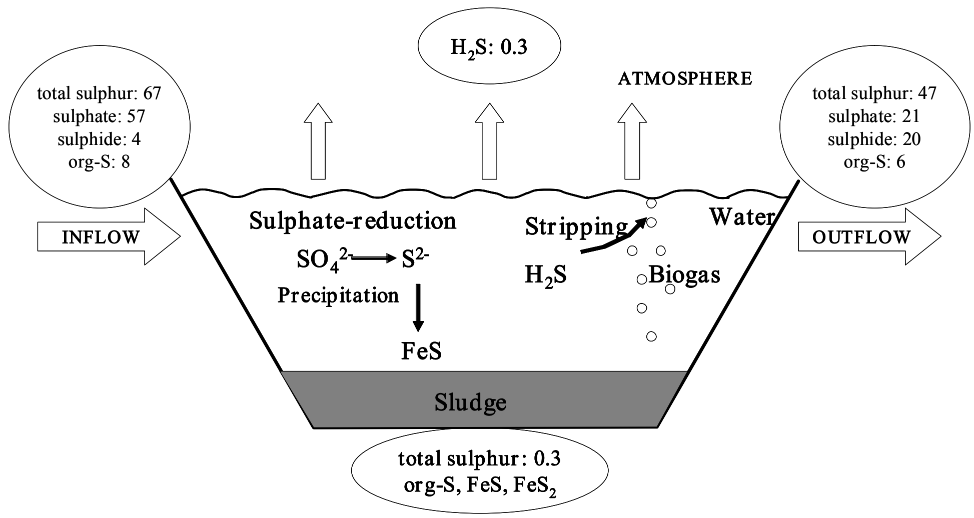

Figure 3 Annual mass balance of sulphur in an anaerobic pond; load expressed in kg S d^{-1} (Paing et al., 2003)

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.

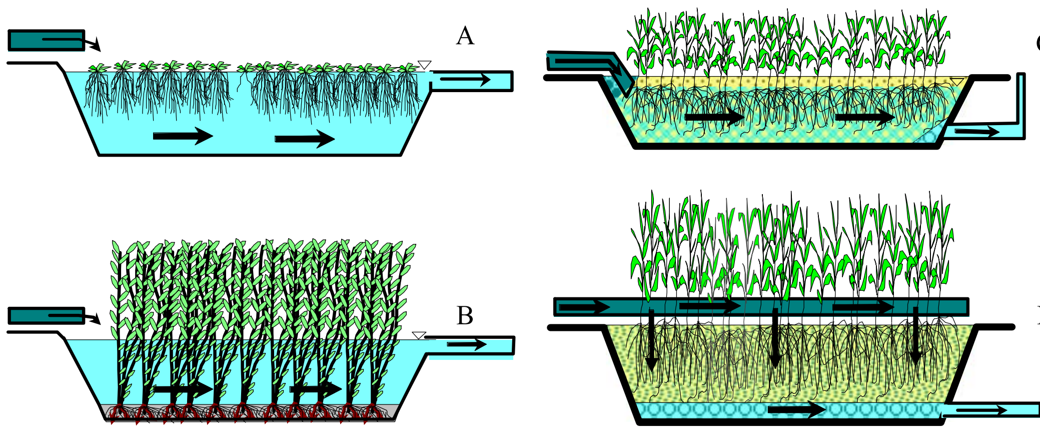

Figure 4 Pond/wetland systems for wastewater treatment (A, pond with free-floating plants; B, horizontal surface flow wetland or pond with emergent water plants; C, horizontal subsurface flow wetland; D, vertical flow wetland).

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:

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

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

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

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

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

- 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 $\mu mol\ g^{-1}d^{-1}$ 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.

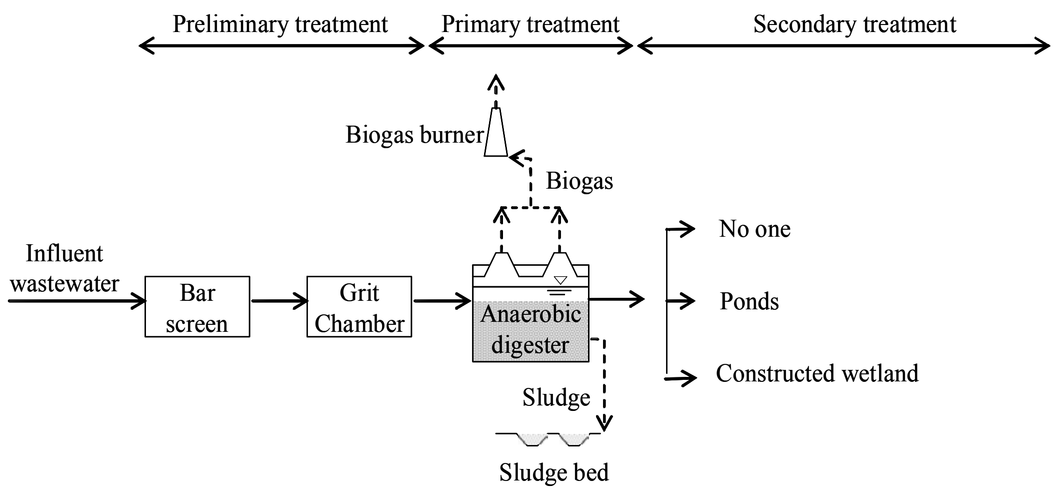

Figure 5 Process flow diagram for a treatment plant designed to meet secondary treatment with constructed wetland.

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.

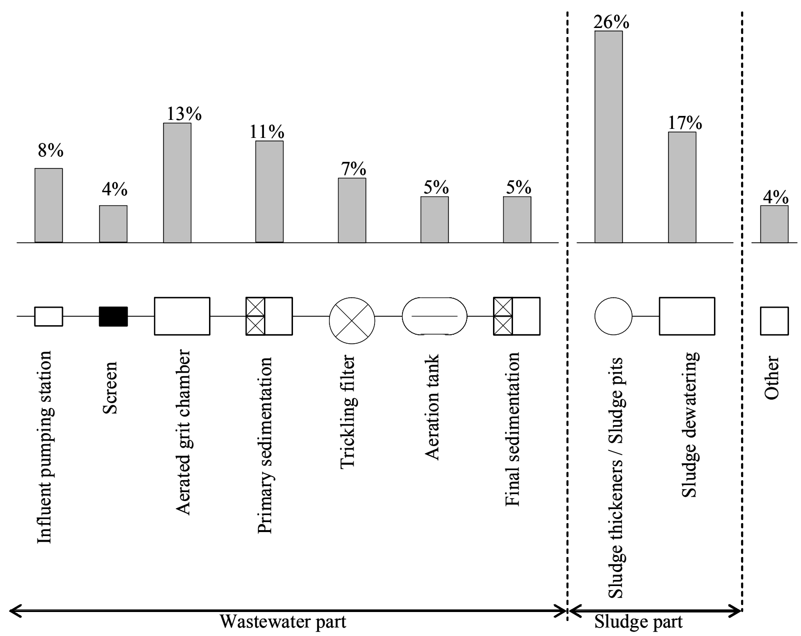

Figure 6 Major sources of odour identified from Frechen's (1998) survey of 100 German sewage treatment works (Figures are percentage of respondents who identified the process as an odour source).

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, ($\mu g\ l^{-1}$)	Range of concentration, ($\mu g\ l^{-1}$)
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.

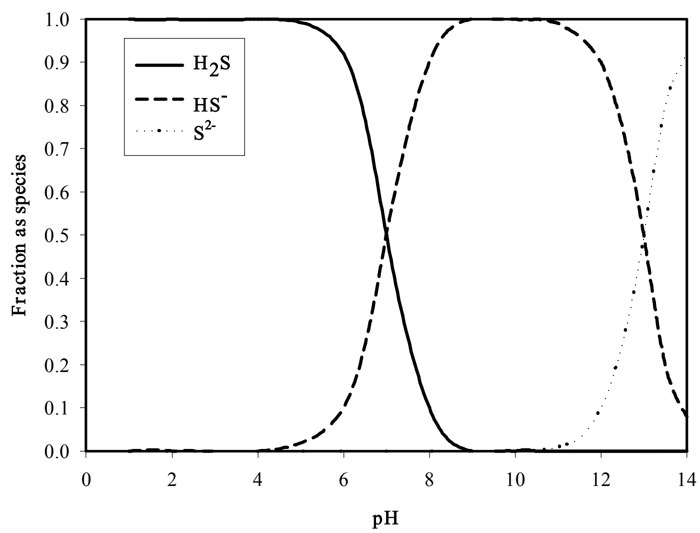

Figure 7 Relative ratio of hydrogen sulphide, bisulphide and sulphide in dependence on pH.

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).

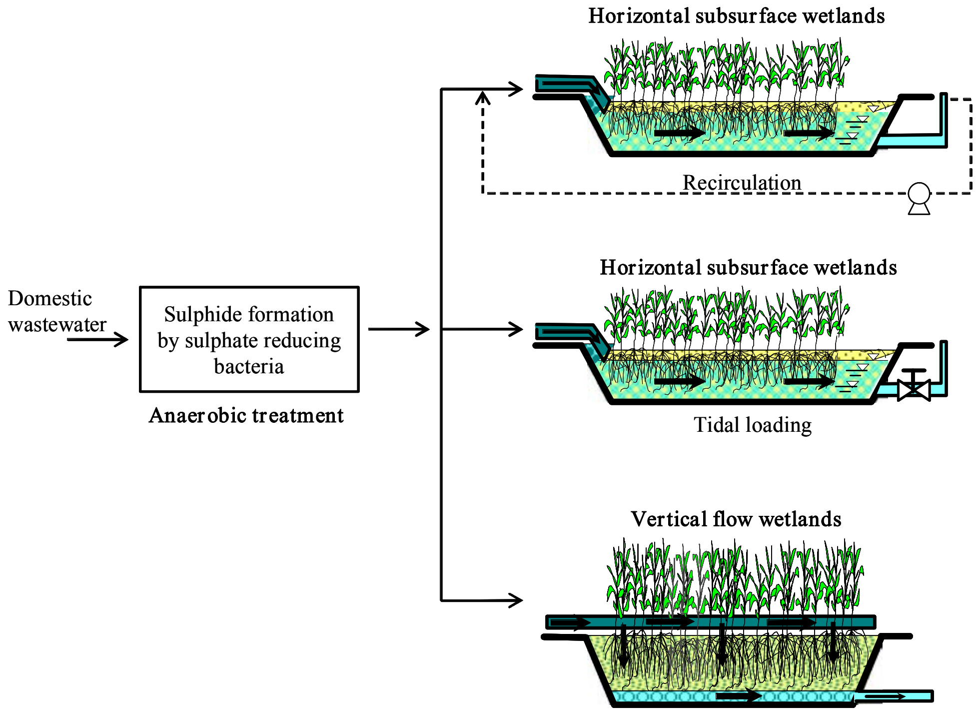

Figure 8 Possibilities for limiting the sulphide intakes to constructed wetlands.

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