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Halophyte filters as saline treatment wetlands

Applications and constraints

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J.J. van der Gaag, M.P.C.P. Paulissen and P.A. Slim

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Abstract

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Purification of wastewater rich in nutrients and organic pollutants is essential for the protection of receiving waters and to enable water reuse. This report investigates the possibilities and constraints of constructed wetlands for treatment of slightly saline wastewater from aquaculture systems. As the body of literature for saline treatment wetlands is relatively small, the reports starts with a summary of processes in freshwater systems. It is then explained that these processes are also present and highly effective in saline treatment wetlands. We conclude with a description of possible vascular plants and macro-algae species for use in saline treatment wetlands. Finally, an assumed case is briefly elaborated.

Keywords: aquaculture, *Aster*, constructed wetland, halophytes, halophyte filters, macro-algae, *Phragmites*, *Salicornia*, saline agriculture, saline effluent, *Spartina*, treatment wetlands, *Typha*, *Ulva*

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Preface

This desk study was carried out in the framework of the IP/OP Strategic Research Programme of Wageningen University and Research Centre.

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Summary

Purification of wastewater rich in nutrients and organic pollutants is essential for the protection of receiving waters and to enable water reuse. Constructed wetlands have proven to be successful in removing a diverse array of pollutants originating from different fresh wastewater sources. As such, constructed wetlands offer a cost-effective, ecologically sound and effective alternative to conventional wastewater treatment.

Contaminant removal from mesohaline (5-18‰) aquaculture effluent is currently a subject of profound interest as well as an increasing body of literature. Marine aquaculture effluent contains particulates, organic matter (including algae), nitrogen and phosphorus. An estimated 70% of N-NH_4^+ is associated with organic solids and 47-84% of P-PO_4^{3-} is particle-bound. The effluent is characterised by a relatively dilute concentration of pollutants. As in constructed freshwater wetlands, contaminants present in brackish or saline wastewater are removed or reduced by a broad range of physical, chemical and biological processes.

Studies on the treatment of mesohaline aquaculture effluent by constructed wetlands show generally high removal rates of suspended solids and phytoplankton (48-99% and 58-95%, respectively). Yet reduction values for Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrogen and phosphorus are highly variable between studies.

For saline wastewater treatment by constructed wetlands ('halophyte filters') in the Netherlands, the use of indigenous species is strongly recommended in order to rule out invasive behaviour. *Phragmites australis*, *Typha* species and vascular tidal-zone plants such as *Spartina anglica*, *Aster tripolium* and *Salicornia* species seem suitable for halophyte filters. Because of their high rates of nutrient uptake, the macro-algae *Ulva lactuca, Laminaria saccharina* (syn. *Saccharina latissima*) and *L. digitata* can be used in aquatic wetlands to treat nutrient-rich wastewater.

Salicornia species, *A. tripolium*, *U. lactuca* and *L. digitata* are useful in that they are edible. Moreover, several studies are under way to develop methods for producing biodiesel from *U. lactuca* and other macro-algae and to produce fodder from *Salicornia* spp., *Suaeda* spp. and *Atriplex* species, in addition to assessing the application of macro-algae in the purification of marine aquaculture effluent.

Further (experimental) research is necessary to gain a better understanding of the performance of vascular plant species and macro-algae at various saline levels and high wastewater loads and to determine the contribution they can make to the removal of contaminants. Research is also needed to find economically viable ways of processing and reusing sludge and plant and algal biomass.

1 Introduction

Purification of wastewater, enriched with nutrients and organic pollutants, is essential for the protection of receiving surface waters and for possible reuse of the treated water (Lin et al., 2002a). Constructed wetlands have proven successful in removing a diverse array of pollutants originating from different wastewater sources (Verhoeven and Meuleman, 1999; Lüderitz and Gerlach, 2002; Faulwetter et al., 2009).

Experience with constructed wetlands has been gained mostly in freshwater systems. Yet demand for ways to treat saline effluent has increased over recent years. Marine aquaculture is an economic sector with a profound interest in treating saline wastewater using constructed wetlands. Constructed wetlands could present a good alternative to conventional wastewater treatment. It is an economical, ecologically sound and efficient means to purify wastewater, which can be subsequently either discharged or reused in a recirculation system.

This report reviews the current state of knowledge regarding the application of constructed wetlands for the treatment of marine aquaculture wastewater. It first describes the various types of wetlands in use today for the treatment of freshwater, along with the major processes and organisms responsible for the removal of contaminants. Subsequently it reviews the literature on the application of constructed wetland systems for treating aquaculture effluent (salinity 3-15‰). The possibilities for the use of constructed saline wetlands in the Netherlands are then explored by first reviewing suitable species of vascular plants and macro-algae for cultivation in a 'halophyte filter'. This is followed by an elaboration of four potential treatment wetland designs employing the suitable species discussed previously. Based on parameter values from the literature, an approximation is made of the required area of constructed wetlands to treat the wastewater from shrimp aquaculture. Finally, conclusions are drawn regarding the applicability of constructed saline wetlands.

2 Wetland systems for freshwater purification

2.1 Natural wetland ecosystems

Wetlands are defined as transitional areas between terrestrial and aquatic systems where the water table is usually at or near the surface of the land, or where the land is covered by shallow water (Cowardin et al., 1979). Soils are water saturated either permanently or at least part of the year (Williams, 2002). The water found in wetlands is static or flowing and can be fresh, brackish or saline (internet site 1). Natural wetlands can be classified into five major types: marine (coastal wetlands), estuarine (including tidal marshes and mangrove swamps), lacustrine (wetlands associated with lakes), riverine (wetlands along rivers and streams) and palustrine (including marshes, swamps and bogs) (Cowardin et al., 1979; internet site 1). These categories differ in water regime, water chemistry and soil type (Cowardin et al., 1979). Wetland ecosystems are distinguished by characteristic plant species and often have a high biological diversity (Kalff, 2002).

Natural wetlands have a great potential for remediating nutrients (Johnston, 1991; Saunders and Kalff, 2001) and pollutants (Rai, 2009) from the water flowing through them, enhancing water quality in downstream surface water bodies. Both natural and constructed wetlands have been purposely loaded with several types of wastewater (municipal, industrial, agricultural, storm water) and used for water purification since the 1950s (Verhoeven and Meuleman, 1999).

2.2 Constructed wetlands

The use of constructed wetlands instead of natural ecosystems for wastewater purification has many advantages. First and foremost it preserves the value of natural wetlands and avoids potential detrimental effects of wastewater application in nature areas. Moreover, constructed wetlands can be designed in such a way that wastewater treatment is optimal, and they can be situated near the wastewater source (Toet, 2003).

As a means of wastewater purification, constructed wetlands have proven successful in removing an array of pollutants originating from different wastewater sources (Lüderitz and Gerlach, 2002; Faulwetter et al., 2009). However, there are still gaps in the understanding of these purification systems. Several studies have explored the applicability of constructed wetlands as a cost-effective alternative to conventional wastewater treatment. These have focused mainly on the use of organic chemicals not normally present in surface or groundwater and on substances that occur naturally in the water, though in the studied cases these were in unusually high concentrations (Imfeld et al., 2009).

The principle of constructed wetlands is based on a combination of physical, chemical and biological processes naturally occurring in wetlands and interrelated with the vegetation, soil and micro-organisms present. The constructed wetland system consists of four major elements: (i) plants, (ii) soil and sediment, (iii) microbial biomass and (iv) an aqueous phase loaded with contaminants. This system is usually extended with a pre-settling basin upstream and discharge ditches or ponds to collect the purified effluent and return it back to the environment downstream (Imfeld et al., 2009).

Wetland plants, mainly helophytes, are of specific importance in the complex interactions between vegetation, soil and micro-organisms involved in purification efficiency (Wießner et al., 2002). Helophytes are plants found in marshy grounds. They root underwater, but have plant parts above the water surface as well. The plants are morphologically and physiologically adapted to flooding (Stottmeister et al., 2003). Via a tissue called 'aerenchyma', resembling connected chambers that act as a gas channel, helophytes are able to supply their inundated root system with oxygen from the atmosphere (Figure 2). The majority of the oxygen is used by the roots for respiration. However, part of the oxygen is continuously released into the rhizosphere by the tips of main roots and by the lateral roots (Brix, 1994; Jackson and Armstrong, 1999). In this way, zones rich in oxygen are created around the roots, compensating for biological and chemical oxygen consumption (Stottmeister et al., 2003). Although several plant species are suitable for wastewater treatment in constructed wetlands, reeds (*Phragmites australis*), types of rushes (*Juncus* spp., *Scirpus* spp., *Bolboschoenus maritimus*) and cattails (*Typha* spp.) are the ones most frequently used (Stottmeister et al., 2003).

Soil-based constructed wetlands can be divided in two basic types: surface-flow wetlands and subsurface-flow wetlands (also known as infiltration wetlands). In the case of surface-flow wetlands (or free water surface wetlands (FWS), Figure 1a), the wastewater flows horizontally over the substrate surface among the vegetation. This type strongly resembles natural wetland ecosystems. Often the system consists of a pre-settling basin and a number of shallow water compartments (water depth 0.2-0.4 m) planted with submerged and emergent macrophytes and floating vegetation (Kadlec and Wallace, 2009). Frequently, the wastewater is mixed with surface water or purified effluent (Verhoeven and Meuleman, 1999). Dikes can be used to control flow and infiltration by expanding the hydraulic retention time (HRT) (Tilley et al., 2002).

Subsurface-flow wetlands are among the most widely used types of constructed wetlands in Europe (Vymazal, 2005). They consist of beds, made of a permeable, granular medium and planted with emergent macrophytes. The beds are usually dug into the ground and lined with an impermeable membrane (a synthetic or clay layer). Subsurface-flow wetlands are categorised in two types, distinguished by the flow direction of the wastewater through the filter medium (i.e. horizontal or vertical) (Vymazal, 2005). In horizontal flow systems (HSSF), the wastewater is maintained at a constant depth and flows parallel to the surface through the granular medium (Figure 1b). The depth of the filter bed is usually 0.6-0.8 m (Vymazal, 2005). In vertical flow systems (VF), the wastewater is distributed over the surface of the filter bed using a pump and drainage tubes on top of the filter medium, possibly after pre-treatment of the effluent in a pre-settling basin (Brix and Arias, 2005). The water percolates downward through the medium (Figure 1c). The infiltration process can be enhanced by embedding drainage tubes in gravel under the filter medium at a depth of 0.6-1.0 m (Verhoeven and Vermeulen, 1999). To secure a high oxygen level in the filter, the filter must not be saturated or covered by water. Wastewater is applied mainly intermittently creating alternately flooded and dry filter conditions (Kadlec and Wallace, 2009). This is distinctly different from horizontal flow systems where wastewater loading is continuous.

For further reading on treatment wetlands we refer to Kadlec and Wallace (2009).

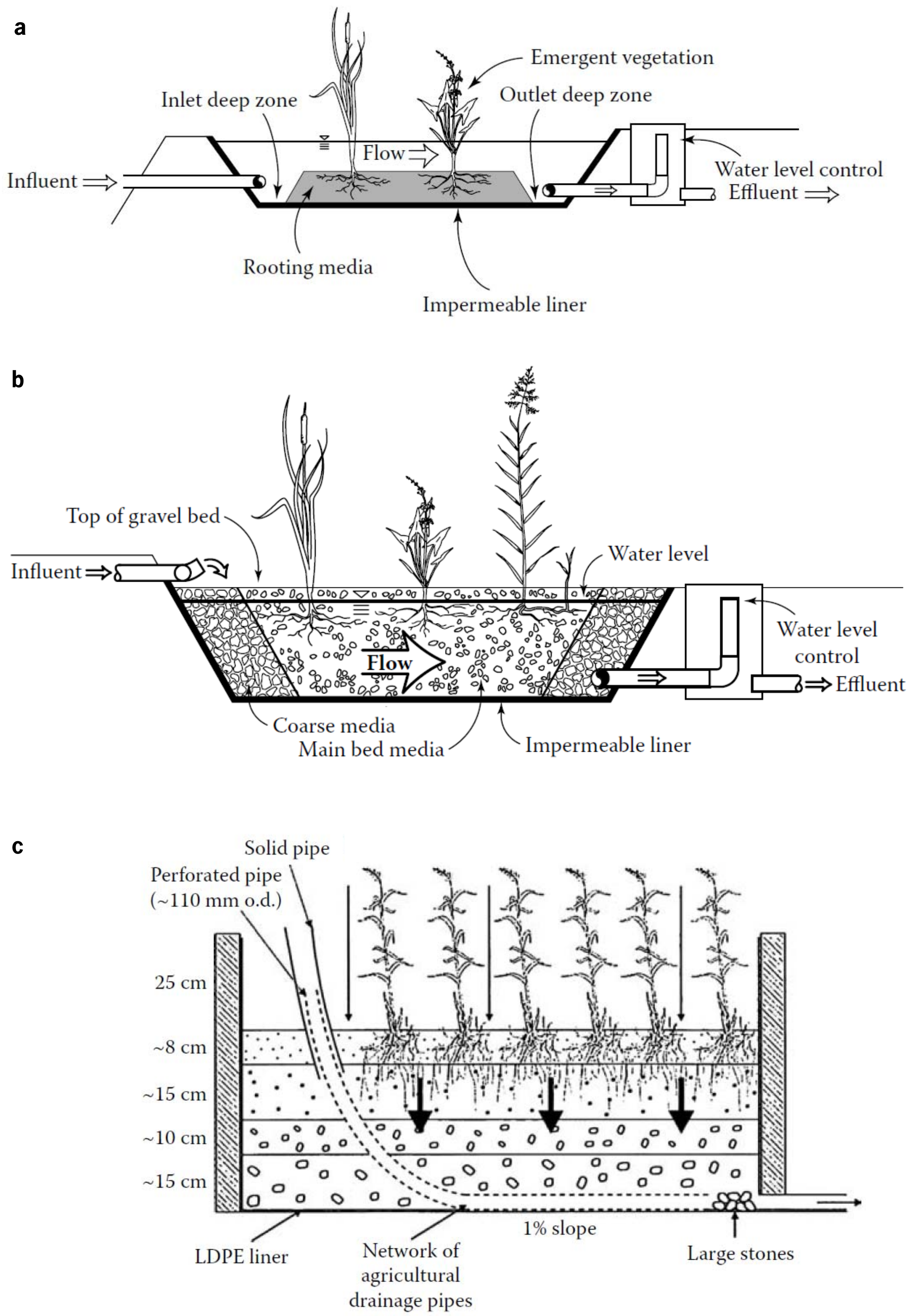

Figure 1

Types of constructed wetlands: 1a. Surface-flow wetland (FWS), 1b. Horizontal subsurface-flow wetland (HSSF), 1c. Vertical subsurface-flow wetland (VF). From Kadlec and Wallace (2009)

2.3 Purification processes in constructed wetlands

In constructed wetlands, contaminants are removed from wastewater or their concentrations reduced by a broad range of processes. The relative importance of a certain process depends on factors such as the constituents of the wastewater, level of pre-treatment, load concentration, type of constructed wetland, operational design (i.e. loading rate, hydraulic retention time, loading regime), filter medium, vegetation type, nutrients already present in the system, desired constituency of wetland effluent and environmental conditions (Verhoeven and Meuleman, 1999; Imfeld et al., 2009). This section enumerates the major removal processes and organisms that play a role in constructed wetlands.

Settlement of suspended solids
Sedimentation of suspended solids occurs when (contaminant) molecules are integrated with particulate organic matter that subsequently settles (Imfeld et al., 2009). This physical process can result in especially high removal rates for suspended organic substances and thus in low downstream levels of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)[1]. Rapid decomposition processes in the water and in upper soil layers also reduce downstream BOD and COD levels (Verhoeven and Meuleman, 1999).

Nutrient uptake by vegetation, phytoaccumulation and other plant functions
Wetland plants can contribute to the storage and removal of nutrients in the system by acting upon microbial, physical and chemical processes and by using nutrients for their own growth. The vegetation in constructed wetlands functions as temporary storage for nutrients. However, large quantities of nutrients can be removed permanently from the system by harvesting part of the vegetation after the senescence of the plants has started and before reallocation of nutrients to the root system occurs (Verhoeven and Meuleman, 1999). If the vegetation is not harvested, a large part of the nutrients incorporated in the plant tissue will again be gradually released to the water by decomposition processes. Furthermore, plants can play a role through accumulation of contaminants in the plant tissue (Imfeld et al., 2009). These contaminants can be removed by harvesting the vegetation.

Apart from nutrient uptake and phytoaccumulation, several other functions of wetland plants influence wastewater treatment in constructed wetlands. The release of oxygen from the roots into the rhizosphere creates oxidised conditions in the otherwise anoxic substrate and stimulates both aerobic decomposition of organic matter and growth of aerobic nitrifying bacteria (Brix, 1994; Van Bodegom et al., 2005). Plants can also release organic substances into the rhizosphere and in this way supply micro-organisms with substrates (Barber and Martin, 1976). Vegetation, further, increases the surface area for particle interception, thereby facilitating sedimentation (Howard-Williams, 1985), and it provides additional surface area for attached microbial growth (biofilm), resulting in increased uptake and conversion of soluble contaminants (Howard-Williams, 1985; Brix, 1994; Brix, 1997; Kadlec and Wallace, 2009). Moreover, plants provide good conditions for physical infiltration by the structure of the macropore system they create, effectively channelling water through the filter bed. Plant root systems stabilise the filter bed surface (especially in surface-flow and horizontal subsurface-flow wetlands) and prevent the formation of erosion channels. In vertical subsurface-flow systems, plants, together with an intermittent loading regime, prevent the filter medium from clogging (Brix, 1994; Brix, 1997). In surface-flow systems, vegetation increases sedimentation of suspended solids by reducing water current velocity, wind-induced mixing and resuspension of settled material (Kadlec and Wallace,

[1] BOD is a measure for the biodegradable amount of organic substances in water (Kim et al., 2001). By means of a BOD_5 test the uptake rate of oxygen by micro-organisms is determined. COD refers to the chemical oxidation of organic compounds and is expressed as the amount of oxygen required to oxidize organic compounds to CO_2, NH_3 and water.

2009). Reduced water current velocity increases the contact time between water and plant surface area. A drawback of a decrease in wind velocity is that it also reduces the aeration in the water column, thereby limiting aerobic processes (Brix, 1997). In open water systems, a plant canopy retards algae growth by diminishing incoming light. Vegetation also insulates the filter against frost during winter, increasing the length of the period of microbial activity during the year. Finally, vegetation in constructed wetlands can provide habitat for wildlife and has an aesthetical value (Brix, 1994).

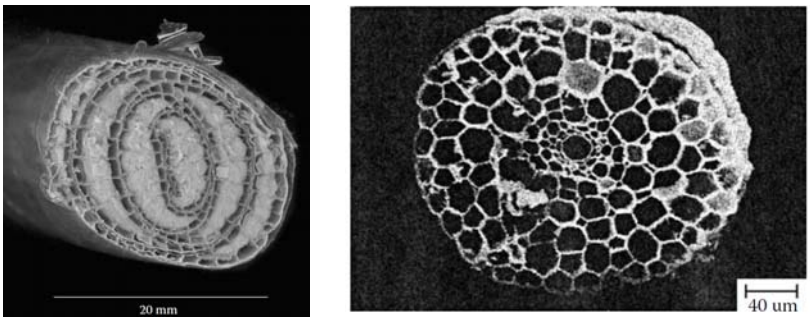

Figure 2
Aerenchyma in a culm of Typha (left) and a root of Phragmites australis (right). From Kadlec and Wallace (2009)

Microbial transformations
Pollutant removal in most constructed wetlands is due primarily to microbial activity (Faulwetter et al., 2009). Microbial transformations remove organic carbon, but are also important in removing nitrogen from the system. Organically derived compounds are broken down by respiration and fermentation into, for instance CO_2, N_2 and H_2O (Faulwetter et al., 2009). The removal of pollutants is dependent on the redox conditions in the constructed wetland. A high redox potential, associated with an oxidised environment, promotes aerobic processes (e.g. nitrification), whereas lower redox potentials are linked to more reduced environments and promote anaerobic processes such as methanogenesis and sulphate reduction (Faulwetter et al., 2009). In wetlands, sharp oxic-anoxic interfaces occur as a result of water table fluctuations, diffusion and advection of oxygen through the water column and soil, and due to active oxygen transport throughout the rhizosphere via the aerenchyma of helophytes (Imfeld et al., 2009). As such, aerobic and anaerobic processes can occur side-by-side.

The biogeochemical cycling of nitrogen in wetlands is complex and involves conversions between different nitrogen species and transfers among different storage compartments (Figure 3; Huang and Pant, 2009). Important microbial processes in nitrogen conversion include nitrogen fixation, microbial assimilation and mineralisation during decomposition, ammonification, nitrification, denitrification, nitrate ammonification and anaerobic ammonium oxidation (anammox) (Huang and Pant, 2009). The nitrogen input in constructed wetland systems consists mainly of organic nitrogen and ammonium (Wood and McAtamney, 1996; Verhoeven and Meuleman, 1999). To remove organic nitrogen from the system, conversion is needed into ammonium (NH_4^+) by a process called ammonification (Verhoeven and Meuleman, 1999).

Nitrification involves two successive aerobic reactions in which NH_4^+ is converted into nitrite (NO_2^-) by ammonium oxidising bacteria (*Nitroso-* genera); subsequently NO_2^- is transformed into nitrate (NO_3^-) by nitrite oxidising bacteria (*Nitro-* genera). In general, nitrification slows with low oxygen concentrations, low moisture content and high C:N-ratios (Hoosbeek et al., 2004).

By denitrification, NO_3^- is converted into atmospheric nitrogen in four successive steps: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. Denitrifying bacteria use organic compounds present in the wastewater as electron donors (Faulwetter et al., 2009). Besides atmospheric nitrogen, small amounts of NO and laughing gas (N_2O, a strong greenhouse gas) are also released. At pH values below 4 the production of N_2 is slowed and the end-product of the denitrification process is N_2O (Van Cleemput et al., 1975). Denitrification takes place under anaerobic conditions in the sediment, in the water column and in the biofilm on the plant surface (Verhoeven and Meuleman, 1999; Toet, 2003). In many wetlands, nitrification rates are much lower than denitrification rates meaning that nitrification can determine the rate of denitrification as well (Verhoeven and Meuleman, 1999). For an optimal denitrification process, both aerobic and anaerobic conditions are required. This can be achieved by using wetland plants that aerate the soil or by using a water regime of alternating dry and wet conditions (Verhoeven and Meuleman, 1999). Interestingly, in surface-flow wetlands, denitrification occurs mainly at night. By day, the process is inhibited due to oxygen produced during photosynthesis by emergent plants and algae (Andersen et al., 1984; Eriksson, 2001).

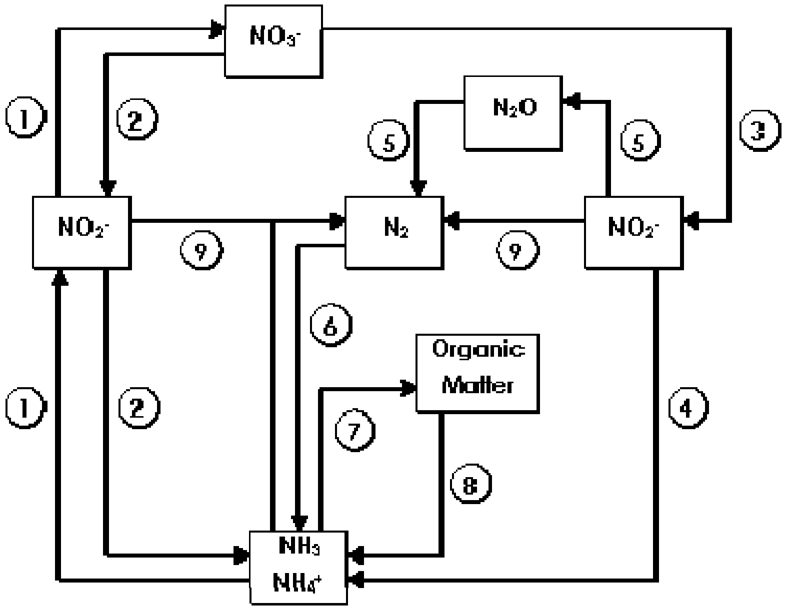

Figure 3

Biogeochemical nitrogen cycle. The figure is derived from Herbert (1999). The numbers indicate the following processes: 1. Nitrification, 2. Assimilatory nitrate reduction, 3. Dissimilatory nitrate reduction, 4. Nitrate ammonification, 5. Denitrification, 6. Nitrogen fixation, 7. Assimilation, 8. Ammonification, 9. Anammox

Anammox is the anaerobic oxidation of ammonium in which NO_2^- and NH_4^+ are converted into N_2 (Jetten et al., 1999). The process was discovered quite recently. Anammox bacteria are autotrophic, unlike classic denitrifiers, which are mostly heterotrophic. The bacteria use nitrite as an electron acceptor. Since anammox bacteria have a slow growth rate (Jetten et al., 2005), a strictly controlled environment and bioreactor arrangement are needed (Lee et al., 2009). Surveys have shown that populations of anammox bacteria are present in freshwater ecosystems and also in wastewater treatment wetlands (Jetten et al., 2005). Anammox bacteria are known to occur in marine sediments as well (Schmid et al., 2007; Van de Vossenberg et al., 2008).

Volatilisation and phytovolatilisation

Via the process of volatilisation contaminants can be directly emitted via the water phase to the atmosphere. Furthermore, some wetland plants are able to transfer pollutants via the transpiration system or aerenchyma to the atmosphere in a process called phytovolatilisation (Imfeld et al., 2009). In this way, volatile anthropogenic chemicals but also product gases created during treatment processes like ammonia (NH_3), hydrogen sulphide (H_2S), nitrogen gas (N_2), nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) may be lost (Kadlec and Wallace, 2009). Volatilisation can lead to air pollution and thus be a kind of pollution swapping.

Physicochemical adsorption and precipitation in the sediment
Removal of the majority of phosphates and heavy metals occurs by adsorption to soil particles (Gopal, 1999). The extent of adsorption depends on the presence of iron, aluminium and calcium in clay minerals or bound to soil organic matter (Verhoeven and Meuleman, 1999). Adsorption capacity is high in the early stages of the constructed wetland but decreases with increasing contaminant addition (Imfeld et al., 2009). As long as the adsorption complex is not saturated the system can act as a sink for the contaminant. The adsorption process is partly reversible depending on the redox conditions (Nichols, 1983). Phosphates bind Fe(III) in aerobic, acid to neutral soils, whereas under anaerobic conditions (i.e. flooding) phosphorus forms complexes with Fe(II) resulting in a release of phosphorus due to less strong adsorption. Adsorption of phosphorus to calcium occurs only under neutral to basic conditions (Verhoeven and Meuleman, 1999). It is important to keep in mind that the addition of wastewater rich in BOD can result in the release of phosphorus already accumulated in the sediment (Gopal, 1999). The microbial decomposition of biodegradable substances decreases the redox potential thereby reducing Fe(III) to Fe(II).

Formation and precipitation of insoluble compounds in the sediment can also occur (Wood and McAtamney, 1996). Phosphate can precipitate with iron, aluminium, calcium and soil particles. This process is slower than adsorption but less susceptible to saturation. Previously adsorbed phosphorus can also precipitate, and in this way new phosphorus can be adsorbed at the sorption site (Verhoeven and Meuleman, 1999).

In case of subsurface-flow systems a filter medium rich in iron, aluminium or calcium can be used to create extra adsorption sites, thereby increasing the amount of bound phosphorus. To remove phosphorus permanently from the system, both for surface-flow and subsurface-flow systems, phosphorus-saturated substrate must be removed (Wood and McAtamney, 1996).

2.4 Nitrogen cycling in brackish to saline environments

In coastal marine ecosystems, the microbially mediated processes that make up the complex nitrogen cycle correspond to the processes that occur in freshwater ecosystems. The nitrogen transformations are performed by a metabolically diverse range of micro-organisms and are strongly influenced by the prevailing physicochemical conditions (Herbert, 1999).

2.4.1 Nitrogen transformations in coastal marine ecosystems

Nitrogen fixation
Rates of nitrogen fixation by heterotrophic and chemolithoautotrophic bacteria have been found to be highest in organically rich sediments. The nitrogen fixation capacity of cyanobacterial mats in salt marshes is considered to be less important as an overall source of fixed nitrogen. Nitrogen fixation appears to be limited in unvegetated coastal marine systems due to the lack of metabolisable carbon. In systems with high nitrogen availability in the sediment and water column, nitrogen fixation provides only a small part of the fixed nitrogen requirements of plants (Herbert, 1999).

Ammonification
Ammonification plays a central role in nitrogen cycling in coastal marine ecosystems (Herbert, 1999). Nitrogenous organic matter is mineralised by a wide range of micro-organisms, such as species from the genus *Pseudomonas* as well as many fungi and actinomycetes. The structure of the organic matter determines whether ammonification is a simple deamination reaction or whether the organic matter has to be broken down

in several complex successive steps. Ammonification rates in unvegetated sediments commonly range from approximately 30 to 160 mg N m^{-2} day^{-1}, whereas rates found in vegetated sediments are 250 to 650 mg N m^{-2} day^{-1} with extremes from 50 to 1125 mg N m^{-2} day^{-1} (Herbert, 1999). The lower ammonification rates found for bare sediments can probably be attributed to the lower organic nitrogen input in these systems. Processes that play a role in mineralisation of organic nitrogen in marine systems, however, are still poorly investigated (Herbert, 1999).

Nitrification

Oxidation of ammonium is very important in shallow coastal ecosystems to create a source of nitrate for denitrifying bacteria (Herbert, 1999). In these systems, species from the genus *Nitrosomonas* are mainly responsible for the first step in the nitrification process, whereas *Nitrobacter* spp. are the principal organisms that conduct the second step in the process. Stephen et al. (1996) demonstrated the existence of marine *Nitrosospira* species in marine sediment samples. These differ from the *Nitrosospira* spp. found in soils. The abundance and diversity of nitrifiers in marine environments is still uncertain (Herbert, 1999).

Nitrification rates (calculated on a yearly basis) are quite similar for different coastal marine and estuarine sediments, ranging from 20 to 40 mg N m^{-2} day^{-1}, with extremes from 2 to 112 mg N m^{-2} day^{-1} (Herbert, 1999). However, seasonal differences in nitrification rate do exist. In some coastal waters, nitrification rates are at a minimum during summer, whereas in other marine systems maxima of nitrification are observed during summer. The activity of nitrifying bacteria in coastal marine sediments is regulated by a number of physicochemical and biological factors such as temperature, NH_4^+ concentration, O_2 concentration, pH, dissolved CO_2 concentration, salinity, presence of inhibitory compounds, light, macrofaunal activity and presence of macrophyte roots (Herbert, 1999). Increased sediment temperature generally results in increased nitrification rates. However, temperature also affects other parameters. An increase in temperature may lead mainly to reduced O_2 solubility. When combined with increased benthic respiration, downward oxygen diffusion is limited to the top 1-2 mm of the sediment. At high ambient temperatures, in sediments rich in organic matter, oxygen availability rather than temperature is probably the factor that limits nitrification most (Herbert, 1999). Nitrification activity can be stimulated by emergent and submersed vascular plants, as they release oxygen into the rhizosphere. Macrofaunal activity can disturb the sediment and significantly alter the spatial gradients for oxygen and NH_4^+ in the sediment (Herbert, 1999).

Denitrification

Denitrification rates in temperate marine environments show distinct seasonal patterns. These are mainly determined by temperature, nitrate concentration, the amount of organic carbon available and oxygen concentration (Seitzinger, 1988). In intertidal and sub-tidal environments denitrification can be inhibited, as oxygen produced during photosynthesis can diffuse into the sediment surface. Denitrification rates reported for coastal marine environments range from 0 to 360 mg N m^{-2} day^{-1} (Seitzinger, 1988). Herbert (1999) reports higher rates of between 650 and 1400 mg N m^{-2} day^{-1}. In general, denitrification rates increase with increasing temperature (Seitzinger, 1988). Studies investigating the effect of oxygen concentration on denitrification consistently show that, in both fresh-water and marine systems, an oxygen concentration of approximately 0.2 mg l^{-1} or less is required for denitrification in water or in sediment (Seitzinger, 1988).

In estuarine sediments, denitrifying bacteria belonging to the genus *Pseudomononas* are most frequently found (Herbert, 1999). Macrophytes and macrofauna play an important role in denitrification in coastal marine systems as well. Since macrophytes can trap easily degradable organic matter and release labile organic carbon from the roots, denitrification can be stimulated. Macrofauna living in burrows in the sediment can influence the exchange of nitrate between the water and the sediment. Shallow burrowers (e.g. *Corophium volutator*) stimulate nitrification, whereas deep burrowers (e.g. *Nereis virens*) usually show a nitrate flux into the burrow, inferring a high rate of denitrification (Herbert, 1999). Macrofauna also influences nitrogen cycling in marine sediments by selectively concentrating organic material as faecal pellets. The pellets form sites of intense microbial activity. Due to this activity, oxygen demand is high, resulting in the formation of reduced microniches. As such, anoxic sites can occur in oxic surface sediments and anaerobic processes, such as denitrification, can occur in close proximity to aerobic processes such as nitrification (Herbert, 1999).

Nitrate ammonification
Denitrification is universally acknowledged as the dominant process of nitrate reduction in most shallow marine ecosystems. The process of nitrate ammonification, however, can be an alternative pathway to reduce nitrate (Herbert, 1999). In marine sediments, heterotrophic bacteria occur that are able to transform nitrate to ammonium. In contrast to denitrification, in the course of which nitrogen is withdrawn from the system due to the diffusion of gaseous end-products to the atmosphere, nitrate ammonification results in nitrogen being retained in the system. Which process of nitrate reduction is dominant depends on several factors. In organically rich sediments, nitrate ammonification rates have been found to be higher than in sediments low in organic matter. The nitrate concentration also plays an important role in determining whether nitrate is reduced to gaseous compounds or conserved as ammonium. At low nitrate concentrations denitrifiers are outcompeted by heterotrophic bacteria that transform nitrate into ammonium, whereas at high nitrate concentrations denitrification is the dominant process (Seitzinger, 1988; Herbert, 1999).

Anammox
All current knowledge on anammox bacteria is derived from species occurring in non-saline water systems. However, the process of anaerobic ammonium oxidation has been demonstrated to be significant in the marine nitrogen cycle (Van de Vossenberg et al., 2008). Anammox activity is detected in a range of marine ecosystems, including in estuarine and coastal sediments. Anammox bacteria may be responsible for 25-50% of nitrogen removal from marine ecosystems (Schmid et al., 2007). The relative contribution of anammox to total N_2 production seems closely related to the sediment reactivity and the availability of nitrate or nitrite in the pore water (Schmid et al., 2007). In the marine environments investigated so far, *Scalindua* is the dominant genus of marine anammox bacteria (Van de Vossenberg et al., 2008). Van de Vossenberg et al. (2008) found that *Scalindua* marine anammox bacteria consumed the same substrates as the non-saline wastewater anammox bacteria, at similar rates, however at lower temperatures. Whether anammox plays a significant role in halophyte filters is uncertain. Further research is necessary to better understand anammox and its contribution in marine ecosystems.

2.4.2 Comparison of nitrification and denitrification in coastal marine and freshwater systems

In aquatic systems, nitrification rates are generally low whereas denitrification is rapid, leading to low nitrate concentrations. Aquatic sediments are characterised by the accumulation of NH_4^+. In littoral sediments of lakes and wetlands, nitrification occurs primarily in two zones: (i) the oxidised rhizosphere and (ii) the water column and oxidised sediment-water interface (Reddy et al., 1989). Nitrification (and denitrification) in aquatic sediments is believed to be controlled mainly by changing oxygen concentrations in the water above the sediment (Rijsgaard et al., 1994). The changing oxygen concentrations are the result of photosynthetic activity. Benthic microphytes occurring in both freshwater and estuarine sediments have a distinct effect on both nitrification and denitrification (Rijsgaard et al., 1994).

Nitrification rates per unit volume in sediments are at least an order of magnitude greater than nitrification rates in the water column. In coastal sediments, for instance, nitrification rates in the sediment are often about 6.7 mg N l^{-1} day^{-1}, whereas in coastal waters, rates range from 3.4 x 10^{-4} to 3.4 x 10^{-2} mg N l^{-1} day^{-1} (Seitzinger, 1988). The volume of sediments in which nitrification occurs, however, is in general much less than the volume of water with nitrification. Rijsgaard et al. (1994) measured nitrification rates of between 0 and approximately 7 mg N m^{-2} day^{-1} in freshwater. Strauss et al. (2000) found that in freshwater systems organic carbon content inhibits nitrification, and that inhibitory effect increased with organic carbon quality. This negative relationship may produce spatial and temporal variability in sediment nitrification rates in aquatic systems. Observation of wastewater treatment systems has revealed that at dissolved oxygen concentrations lower than approximately 2.5 mg l^{-1}, nitrite oxidation is inhibited, leading to its accumulation (Paredes et al., 2007).

Figure 4 shows the denitrification rates measured in sediments from rivers, streams and coastal marine systems as summarised in Seitzinger (1988). In general, coastal marine sediments show a greater range of denitrification rates than found in lake or river sediment. Higher rates of denitrification are found for systems that receive substantial anthropogenic nutrient loading (e.g. sewage water). A few studies, mainly relating to lakes, report denitrification rates in the water column that are considerably lower than the rates found for the sediment. This difference is caused by low oxygen conditions, rapid nitrification rates and abundant supply of organic matter in the sediment. The major source of nitrate for denitrification in most lake sediments is nitrate produced in these sediments and not nitrate diffusing into the sediments from the overlying water (Seitzinger, 1988). In freshwater sediments, a larger percentage of the mineralised nitrogen appears to be removed by denitrification than in marine sediments. In lakes and streams, the sediment-water nitrogen flux due to denitrification ranges from 74% to 100%, whereas in coastal and estuarine systems, this flux accounts for only 15-75% (Seitzinger, 1988).

In marine sediments, sulphate reduction is the predominant pathway of anaerobic metabolism, whereas in freshwater sediments methanogenesis predominates (Seitzinger, 1988). Sulphate occurs widely in seawater, in sediment and in waters rich in decaying organic material (internet site 2). The reduction of sulphate is an anaerobic process in which sulphate is converted into hydrogen sulphide (H_2S) by sulphate-reducing bacteria. Much of the hydrogen sulphide reacts with metal ions in the water to produce metal sulphides, such as ferrous sulphide (FeS). Sulphide completely inhibits nitrification at concentrations between 0.9 and 40 μM. Denitrification is not inhibited by sulphide at concentrations as high as 300 μM. However, the process can be indirectly inhibited by sulphide if nitrification is suppressed (Seitzinger, 1988).

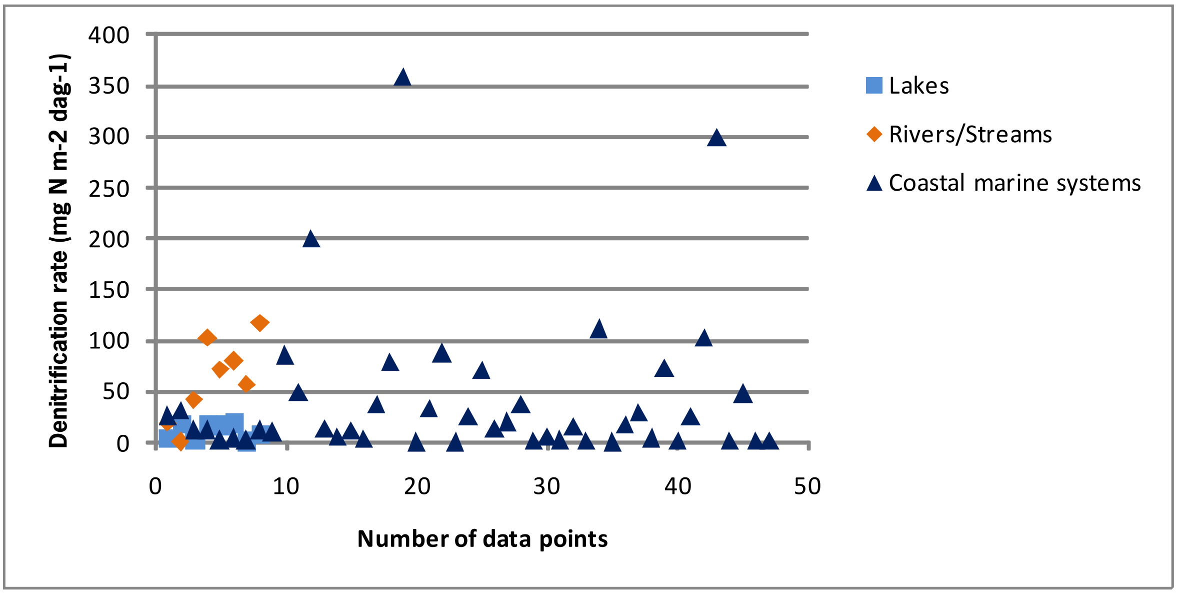

Figure 4

Denitrification rates reported for sediments in lakes, rivers and streams, and coastal marine ecosystems. Data are extracted from Seitzinger (1988)

2.5 Purification efficiency of constructed wetlands

Hydraulic variables

The efficiency of contaminant removal in constructed wetlands depends on hydraulic variables such as hydraulic loading rate (HLR) and hydraulic retention time (HRT) (Toet et al., 2005). The HLR (m day^{-1}) is defined as the ratio of water flow rate from the fishpond to the constructed wetland (m^3 day^{-1}) and the wetland surface area (m^2), whereas the HRT (day) is calculated as the wetland surface area (m^2) times the water depth (m) times the porosity (-) of the constructed wetland(s) divided by the water flow rate (Lin et al., 2005; Toet et al., 2005). Both variables affect the contact time between the wastewater and the wetland. The HRT can be increased by decreasing the HLR or increasing the water depth (Toet et al., 2005).

Redox conditions

With the exception of the aerobic patches in the proximity of helophyte roots, anaerobic processes predominate in subsurface-flow systems, whereas aerobic processes usually prevail in surface-flow systems (Stottmeister et al., 2003). In subsurface-flow systems, vertical flow (VF) wetlands, which are not permanently water saturated, have better aeration of the filter bed compared to horizontal flow wetlands (HSSF). Therefore, VF systems generally operate under more oxidised conditions, favouring aerobic microbial populations and pollutant removal mechanisms, whereas HSSF systems typically favour anaerobic populations (Faulwetter et al., 2009). Unlike VF wetlands, which produce nitrified effluents, HSSF systems often have very limited nitrification capabilities (Brix and Arias, 2005; Kadlec and Wallace, 2009; García et al., 2010). High oxygen levels in VF wetlands also lead to high removal of BOD and COD (Cooper, 1999). The horizontal and vertical flow systems can be combined to form hybrid wetlands (Vymazal, 2005). With the use of different wetland types, redox conditions can be manipulated causing a shift in the dominance of various microbial functional groups (Faulwetter et al., 2009). In this way, processes in the systems can be made complementary, resulting in higher removal efficiency (Vymazal, 2005) and a reduced surface area requirement (Brix, 1994).

Contaminant removal

Surface-flow wetlands are most commonly used for processing secondary or tertiary treatment effluents[2] (Kadlec and Wallace, 2009). Subsurface-flow systems are mainly designed to treat heavier polluted primary settled wastewater, although they are also employed to improve the quality of secondary effluents (García et al., 2010). Surface-flow wetlands generally have a lower pollutant removal capacity than subsurface-flow wetlands, due to the smaller interface between sediment and wastewater. However, surface-flow wetlands cost less, due to their simpler construction and operation (Table 1).

The slow moving waters in surface-flow wetlands often permit time for suspended solids to physically settle. The velocity of the incoming particles together with the depth of the wetland give an estimate of the settling time and travel distance of the solids. Figure 5 shows processes that affect particulate removal and generation in surface-flow wetlands. In fully vegetated wetlands, the litter layer created by macrophytes prevents trapped sediments from becoming resuspended in the water column. Furthermore, submerged plant biomass traps particles in sheltered microzones, again reducing the potential for resuspension. Sediments might also collide with or be intercepted by immersed vegetation (Kadlec and Wallace, 2009). Faunal activity, the production of oxygen by algae, and the production of methane in anaerobic zones can cause resuspension of particles that

[2] Primary wastewater treatment uses sedimentation to remove a portion of the suspended solids and organic matter from the wastewater. Secondary wastewater treatment includes the removal of biodegradable organic matter and suspended fine solids. In tertiary wastewater treatment, contaminants not removed by primary and secondary treatments (e.g. nutrients and residual suspended solids) are removed by biological or physical-chemical treatment processes (Tchobanoglous et al., 2003).

were already stabilised (Kadlec and Wallace, 2009). Moreover, during the process of wastewater treatment, particulate matter might be produced by chemical reactions and biota present in the wetland. Algae can be a major generator of suspended solids (Kadlec and Wallace, 2009).

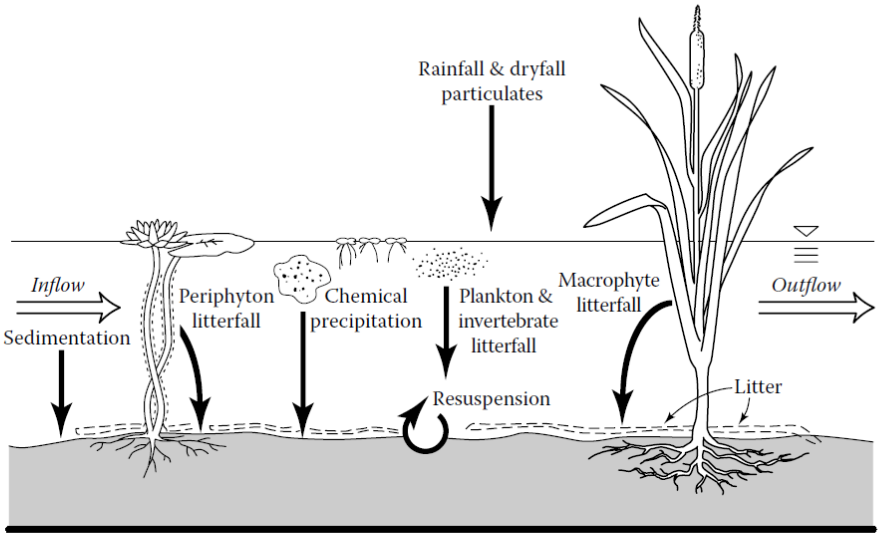

Figure 5

Processes affecting particulate removal and generation in surface-flow wetlands (FWS). From Kadlec and Wallace (2009)

Pre-settling basins placed before the surface-flow wetland as a first compartment in the constructed system can trap the fastest settling fraction of suspended material. Accumulated deposits can be removed more easily from these than from the surface-flow wetland. Kadlec and Wallace (2009) found settling basins occupying 15% of a wetland area to account for 94-97% of the solids removal.

Like in surface-flow wetlands, trapping and retaining organic substances and suspended solids in influent is generally very high in both HSSF and VF systems (Vymazal, 2005; Kadlec and Wallace, 2009). HSSF wetlands, however, have an advantage over VF wetlands with respect to suspended solids removal and removal of bacteria (Cooper, 1999). However, the accumulated material often reduces the hydraulic conductivity of the wetland to some degree (see also the section below on clogging). In contrast to surface-flow wetlands, resuspension mechanisms are minimal in subsurface-flow systems (Kadlec and Wallace, 2009). Most suspended solids in HSSF and VF wetlands are removed through sedimentation and filtration or interception. In VF wetlands nitrogen removal is more efficient and the adsorption of phosphorus is promoted more than in HSSF systems (Toet, 2003). In hybrid systems consisting of a surface-flow wetland followed by a subsurface-flow wetland, most suspended solids are presumably removed in the surface-flow wetland (possibly in combination ith a pre-settling basin), resulting in a well-oxygenated effluent entering the subsurface-flow wetland, where nitrification is stimulated. This also reduces clogging in subsurface-flow wetlands (Lin et al., 2002a).

Compared to surface-flow wetlands, subsurface-flow systems are much less dependent on plants to sustain their treatment processes. The HRT and the duration that the wastewater is in contact with the plant roots affect the significance of the plants in the removal and breakdown of pollutants (Stottmeister et al., 2003). In HSSF wetlands with long hydraulic retention times, plants clearly affect the removal of contaminants. In intermittently loaded VF systems, which usually have short hydraulic retention times, plants play a subordinate role in pollutant removal (Stottmeister et al., 2003). Nutrient removal by plant assimilation and uptake is reported to be between 10% and 30% of total nitrogen and total phosphorus retention (Schulz et al., 2003). Bachand and Horne (2000) found bacterial denitrification rather than plant uptake to be the main mechanism for nitrate removal. Nutrient removal by harvesting aboveground plant biomass is often insignificant relative to the loading in wetland systems, yet in low-loaded systems it can be of importance (Brix, 1997). Emergent macrophytes differ in their nutrient uptake capacity but have higher uptake capacities than submerged macrophytes (Brix, 1997). Ammonium removal in subsurface-flow wetlands varies with plant species (Faulwetter et al., 2009). It must be kept in mind, however, that not all nutrients removed by plants from the wastewater can be removed from the system by harvesting aboveground plant biomass. In one-year-old stands, belowground plant parts of macrophytes such as *Phragmites australis* and *Typha* species account for some 35-50% of the total growth and up to 75% or more in older stands (Gopal, 1999).

Table 1
Comparison of a number of parameters for constructed wetland types: surface-flow wetland (FWS), horizontal subsurface-flow wetland (HSSF) and vertical subsurface-flow wetland (VF). Number of (*) increases with the importance of the parameter or process

	Wetland type		
Parameter/Process	**FWS**	**HSSF**	**VF**
Loading	continuous	continuous	intermittent
Wastewater quality	secondary/tertiary	primary	primary
Flow depth (m)	0.2-0.4	0.6-0.8	0.6-1.0
Prevailing redox conditions	aerobic	anaerobic	aerobic
Dominant N-removal process		denitrification	nitrification
Resuspension	**	*	*
Clogging	-	**	***
Habitat for wild life	**	*	*
Cost	*	**	**
Area	***	**	*

Clogging

The propensity of the filter bed media to clog is an important operational consideration. Both horizontal and vertical subsurface-flow systems are susceptible to clogging, restricting the flow of water through the bed. Resting periods are essential to allow the filter beds to recover from the clogged conditions (i.e. for the hydraulic conductivity to return to original values) via drying and cracking of the medium (Kadlec and Wallace, 2009).

In HSSF systems, wastewater is supplied via an inlet and passes through the filter medium on a more or less horizontal pathway until it reaches the outlet (Vymazal, 2005). The hydraulic conductivity of the inlet zone can be reduced by various mechanisms, including accumulation of mineral sediments associated with suspended solids in the wastewater, accumulation of particulate organic matter within the inlet zone, formation of chemical precipitates within the filter medium, and formation of microbial biofilms (Kadlec and Wallace, 2009). Due to the clogging mechanisms, high cross-sectional loadings result in a greater length of the filter bed operating as an overland flow system. The length of overland flow can be reduced by minimising the cross-sectional loading and by using a coarser medium material in the inlet zone. However, even well-designed HSSF systems require inlet zone maintenance about once every ten years to avoid the development of large areas of overland flow. Filter media replacement is currently the most common method of inlet zone maintenance (Kadlec and Wallace, 2009).

In VF wetlands, wastewater loading is concentrated at the discharge apertures of the drainage tubes. This causes a non-uniform distribution on the filter surface of the wastewater, and thus of total suspended solids and organic matter. These accumulated solids can clog soil pores and result in a loss of hydraulic conductivity which can rapidly lead to flooding of the filter bed. The organic loading applied to the VF system appears to play a large role in the clogging process (Kadlec and Wallace, 2009). To avoid soil clogging in constructed wetlands for the treatment of municipal sewage wastewater, Müller (2000, in Schulz et al., 2003) advised a maximum of 15 g total suspended solids m^{-2} day^{-1}.

3 Constructed wetlands for saline water purification

3.1 Aquaculture effluent treatment: facts and figures

The main fish species and crustaceans cultivated in aquaculture worldwide are catfish (*Siluriformes*), trout (*Salmoniformes*) and shrimp (Kadlec and Wallace, 2009). In the Netherlands, pilot aquaculture experiments have been started in which sole (*Solea solea*) is cultivated together with shellfish (mainly *Ruditapes philippinarum* and *Mytilus edulis*). In the project 'Zeeuwse Tong', surplus nitrogen and phosphorus are estimated to be 6.4 and 14 kg ha^{-1} yr^{-1}, respectively, and the aquaculture effluent discharge is estimated to be 640-900 m^3 ha^{-1} day^{-1}. Salinity is estimated at approximately 30‰ (pers. comm. Sander Ruizeveld de Winter).

Aquaculture effluent is typically produced when ponds or basins are drained during harvest or when pond water is renewed (Lin et al., 2010). In some aquaculture systems in which water is recirculated, the basin water is continually renewed (Lin et al., 2003; Lin et al., 2005). Aquaculture effluent contains particulates, organic matter, nitrogen (nitrate and ammonium) and phosphorus and is characterised by a relatively dilute concentration of pollutants and a large volume of discharge (Lin et al., 2010). An estimated 70% of $N\text{-}NH_4^+$ is associated with organic solids, and 47-84% of $P\text{-}PO_4^{3-}$ discharged from intensive aquaculture systems is particle-bound (Lin et al., 2002a). Table 2 shows the concentrations of several contaminants in shrimp aquaculture effluent for four aquaculture farming locations in Thailand. Effluents originating from trout farms are 20 times more dilute than medium-strength municipal wastewater and are less contaminated than even municipal secondary treatment criteria (Kadlec and Wallace, 2009). In general, evaluation studies indicate that constructed wetlands are an ecologically attractive and economical method for treating salmon and trout farm effluents to reduce solids and phosphorus (Kadlec and Wallace, 2009).

Table 2
Quality of shrimp pond water (Thailand). Data derived from Sansanayuth et al. (1996)

Contaminants (mg l^{-1})	**Aquaculture farming locations**			
	Rayong	Sonkla	Chachengsao	Chuntaburi
Suspended solids	64	64	162	88
BOD	12	-	26	21
Total organic carbon	-	-	65	40
Total N	6.7	2.7	12	8.2
Total P	0.5	0.3	3	0.3

Constructed wetlands can efficiently remove the major pollutants from fishpond effluent, including suspended solids, organic matter, nitrogen, phosphorus and phytoplankton (Lin et al., 2003). Regarding intensive shrimp culture, several studies have examined recirculating aquaculture systems (RAS) which incorporate constructed wetlands to treat aquaculture effluent (Tilley et al., 2002; Lin et al., 2003; Lin et al., 2005). A recirculating aquaculture system allows for intensive culture with limited pollutant discharge, thereby reducing water and land usage and minimising adverse environmental impacts (Lin et al., 2005). In such a system, a culture tank or fishpond is linked to a constructed wetland unit. The effluent from the wetland is collected and recycled back into the culture tank. The construction of the wetland unit differs between studies, with varying degrees of integration in the natural environment. Lin et al. (2005) describes a wetland unit in Taiwan established in a concrete basin[3] and consisting of a settling cell, a surface-flow wetland (FWS) followed by a horizontal subsurface-flow wetland (HSSF) and a sump (Figure 6). Water from the culture tank flowed continuously to the wetland unit by the force of gravity with the flow rate controlled by a gate valve. The floor of the settling compartment was sloped at approximately 14% toward the inlet end. The effluent could flow through a perforated wall from the settling compartment to the FWS wetland. The two wetland types were also separated by a perforated wall. The FWS wetland had a 30 cm layer of local soil (silt loam) at the bottom, whereas the HSSF wetland contained 80 cm of river gravel (porosity 45%). The water level remained constant throughout the study, at 40 cm and 65 cm, respectively, for the FWS and HSSF wetland. The FWS wetland was elevated 30 cm above the HSSF wetland. The treated effluent was re-aerated in the sump before being pumped back into the culture tank. The water used in the culture tank was brackish groundwater (3‰ salinity).

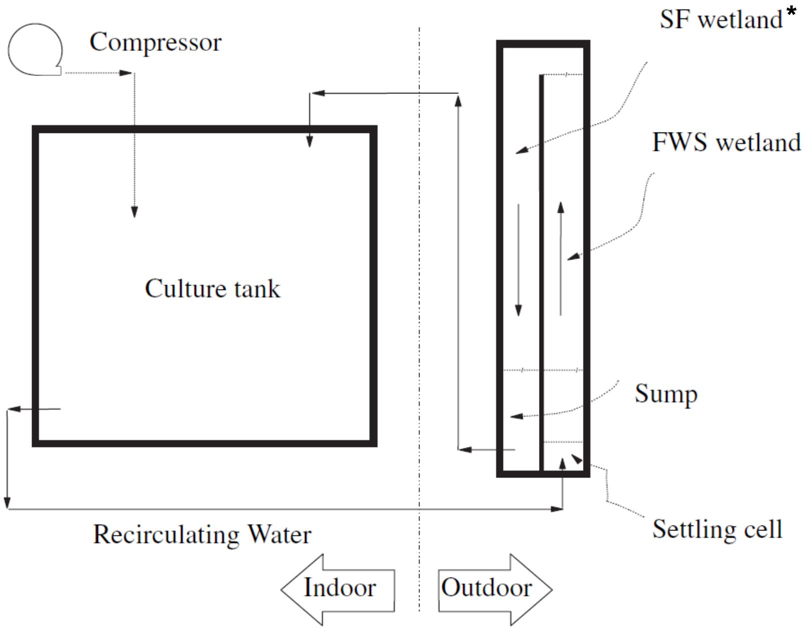

Figure 6
*Schematic arrangement of the recirculating aquaculture system described in Lin et al. (2005). The figure is adapted from Lin et al. (2005). *) SF wetland = HSSF wetland*

Lin et al. (2005) mention that the FWS was planted with *Typha angustifolia* and the HSSF with *Phragmites australis*, both in an initial density of 6 plants m^{-2}. The macrophytes showed rapid growth. After approximately 10 months plant density had increased to more than 90 plants m^{-2} for both wetland compartments. Tilley et al. (2002) reports on a two-year study of a mesohaline (salinity 3-15‰) recirculating system originally planted with ten salt-tolerant wetland plant species[4], but which became dominated by *Typha latifolia* in the second year of the experiment: the macrophyte came to occupy nearly two-thirds of the wetland area. Tilley et al. (2002) found that emergent macrophytes, like *T. latifolia*, can mediate salinity fluctuations. This is presumably due to the diminishment of strong winds and reduced water vapour saturation deficits, both of which limit evaporation

3 Cast iron vessels lined with impermeable plastic liners are also used (Lin et al., 2002 and 2003).
4 These ten plant species were included: *Ruppia maritima, Chara* spp., *Pithophora* spp., *Nymphaea odorata, Hydrochloa caroliniensis, Typha latifolia, Juncus effusus, Sesbania drummondii, Borrichia frutescens* and *Avicennia germinans*.

rates. According to Tilley et al. (2002) the plant diversity of the constructed wetland (and thus probably also its ecological resilience) can be increased by applying ample seed and using a flooding regime that mimics natural wetland conditions.

For a recirculating aquaculture system, removal of solids, organic matter, ammonium and nitrate is crucial (Lin et al., 2005). Removal of algae is also essential in treatment of aquaculture wastewater. Significant algae growth occurs in outdoor fishponds, and although algae can contribute to contaminant removal by nutrient uptake and by stimulating oxidative processes in the ponds, large algae concentrations harm cultivated aquaculture species by increasing the pH level. Furthermore, high algae concentrations in pond effluent increase oxygen demand in the receiving waters due to microbial degradation (Lin et al., 2002a). As a measure of the algae biomass, the chlorophyll-a concentration ($\mu g\ l^{-1}$) is used. To achieve sufficient pollutant removal, constructed wetlands typically require a low HLR and high HRT (Lin et al., 2005). A longer residence time generally allows the area of a constructed wetland to be reduced while achieving similar treatment efficiencies. However, greatly increasing the HRT (to more than 15-20 days) can result in other water quality problems, such as high salinity due to more evapotranspiration and extremely low dissolved oxygen concentrations due to increased soil microbial respiratory activity (Tilley et al., 2002). Typical HRT and HLR ranges suggested for the design of constructed wetlands for wastewater treatment are 4-15 days and 0.014-0.047 m day^{-1}, respectively (Lin et al., 2003). In a recirculating aquaculture system, the wetland compartments usually require a low HRT to achieve a high percentage of removal of (toxic) contaminants and maintain the appropriate water quality in the culture tank (Lin et al., 2003). Increasing hydraulic loads supports the development of aerobic conditions in the constructed wetland, while hindering denitrification processes (Schulz et al., 2003).

Table 3 presents an overview of results concerning contaminant removal and system conditions from several studies on aquaculture wastewater treatment. Most of the studies were conducted in Taiwan, though there are also examples from Thailand, Texas and Germany. In most studies, wetland units consisted of a surface-flow wetland followed by a subsurface-flow wetland. The HLR and HRT in these systems were 0.023-5.143 m day^{-1} and 0.06-10 days, respectively. The aquaculture species cultivated was shrimp in most cases. The literature does not always mention the salinity of the aquaculture wastewater, however, the values that were recorded refer to mesohaline conditions. *Phragmites australis* and *Typha* species were commonly the dominant wetland vegetation, and mixtures of (rather) salt-tolerant species were also used. Ratios of constructed wetland to fishpond area reported in Table 3 are both higher and lower than 1, indicating wetland sizes being 0.005-1.67 times that of the pond area for treatment of polluted fishpond effluents. Box 1 presents a way to estimate the area of the constructed wetland required to meet a given target outlet concentration for a given inlet concentration.

The studies mentioned in Table 3 show high removal of suspended solids and phytoplankton (48-99% and 58-95%, respectively). Lin et al. (2002b) demonstrated that the removal efficiencies of suspended solids decreased with increasing HLR. Regarding recirculating aquaculture systems, Lin et al. (2002b and 2003) showed efficient nitrate removal (68-100%), whereas Lin et al. (2005) and a comparative study by Schulz et al. (2003) demonstrated poor performance in nitrate removal or even an increase in nitrate from influent to effluent. The increase in nitrate indicates that nitrification processes were successful, but denitrification was inhibited. The differences in nitrate removal between the studies mentioned can presumably be explained by differences in HLR. In Lin et al. (2002b and 2003), HLR was less than or equal to 0.3 m day^{-1}, whereas in the latter studies HLR values were more than 1.5 m day^{-1}. High HLR reduces the contact time for nitrate and thus limits denitrification. Another explanation for the poor nitrate removal could be the lack of organic compounds, as denitrifying micro-organisms use these as electron donor for the denitrification process (Lin et al., 2005). Reductions of total phosphorus and $P\text{-}PO_4^{3-}$ from the wastewater in the studies in Table 3 ranged from 38% to 68% and 32% to 71%, respectively. The low removal rates found by Lin et al. (2003 and 2005) may be the result of high $P\text{-}PO_4^{3-}$ loading rate (2.56 g m^{-2} day^{-1}). Lin et al. (2002b) found $P\text{-}PO_4^{3-}$ removal efficiencies to be inversely related to hydraulic loading rate. Removal of phosphorus can be improved by creating calm water conditions so that suspended solids can settle, which in turn promotes adsorption of phosphorus to suspended particles (Tilley et al., 2002). With respect to receiving-water-quality objectives, phosphorus is the most constraining element to remove from freshwater aquaculture farms (Kadlec and Wallace, 2009). Reduction values for BOD and COD are highly variable between studies. Sansanayuth et al. (1996) showed BOD removal values of about 95%, whereas other studies reported values below 55%.

Several aspects can affect removal efficiencies of constructed wetlands and have to be taken into consideration. Logically, constructed wetlands have start-up behaviour due to, for instance, incomplete plant cover, and nutrient removal processes that have not yet reached a steady state. Reports suggest that from three to four months up to two years is required to obtain a complete plant cover in constructed wetlands (Lin et al., 2002b). The system described in Lin et al. (2002b) required two to three months to reach a consistent nitrogen removal performance level for the FWS wetland and one month for the subsurface-flow wetland. Significant phosphate removal was achieved after about three months in the FWS wetland, and immediately after wastewater application in the subsurface-flow wetland. Wetland performance can also change between and within years due to shifts in temperature, seasonal variation, and changes in vegetation composition (succession) and organic loadings (Kadlec and Wallace, 2009). The long-term impact of wastewater application on the functioning of the filter must also be taken into account.

Box 1. Estimating the constructed wetland area required

To estimate the wetland treatment area required for wastewater treatment to meet a given target outlet concentration for a given inlet concentration, the following equation can be used (Lin et al., 2005):

$$A_w = \frac{Q(\ln C_i - \ln C_e)}{k\varepsilon h_w}, \qquad \text{Eq. 1}$$

where Q is the flow rate of the wastewater through the constructed wetland (m^3 day^{-1}), C_i is the influent pollutant concentration (mg l^{-1}), C_e is the effluent pollutant concentration (mg l^{-1}), k is the first order removal rate constant (day^{-1}), ε is the porosity of the wetland and h_w is the water depth of the constructed wetland (m).

This equation omits the background pollution concentration and assumes a steady flow. The removal constant k has to be determined for a specific wetland for each pollutant individually. Tilley et al. (2002) present an equation to calculate k taking into consideration the background concentration of the pollutant. The constant k can also be determined by linear regression analysis of C_0/C_i versus the nominal HRT, with C_0 as the target concentration (mg l^{-1}). Values for k mentioned in the literature show a large variability.

In a recirculating aquaculture system, Q can be derived from (Lin et al., 2005):

$$Q = rA_t h_t, \qquad \text{Eq. 2}$$

where r is the recirculating ratio (i.e. the ratio of daily flow of recirculating water to total water in the culture tank, day^{-1}), A_t is the surface area of the culture tank (m^2) and h_t is the water depth of the culture tank (m).

Table 3

System parameters and contaminant removal found in literature concerning aquaculture wastewater treatment. RAS is Recirculating Aquaculture systems, FWS is surface-flow system, HSSF is horizontal subsurface-flow system, SF is subsurface-flow system, FM is surface-flow system with floating macrophytes. A_w:A_t is the ratio of wetland area to tank or fish pond area. BOD is Biochemical Oxygen Demand, COD is Chemical Oxygen Demand, TSS is total suspended solids, TP is total phosphorus, P-PO_4 is phosphate P, TN is total nitrogen, TIN is total inorganic nitrogen, N-NO_2 is nitrite N, N-NO_3 is nitrate N, N-NH_4 is ammonium N, and Chl-a is chlorophyll-a

Reference	**Lin et al. (2003)**	**Lin et al. (2005)**	**Lin et al. (2005)**	**Lin et al. (2010)**	**Lin et al. (2010)**	**Lin et al. (2010)**	**Tilley et al. (2002)**
Wetland type	RAS FWS-SF	RAS FWS-SF	RAS FWS-SF	RAS FM-SF	RAS FM-SF	RAS FM-SF	FWS
A_w:A_t	1.67	0.48	0.48	0.086	0.086	0.086	0.95
HLR (m day^{-1})	0.3	1.54	1.95	4.51	4.41	2.92	0.177
HRT (day)	0.5 (FWS) 0.26 (SF)	0.096 (FWS) 0.108 (SF)	0.067(FWS) 0.096 (SF)	0.189	0.193	0.293	1
Aquaculture species	*Litopenaeus vannamei*	*Litopenaeus vannamei*	*Litopenaeus vannamei*	*Litopenaeus vannamei*	*Litopenaeus vannamei*	*Litopenaeus vannamei*	*Litopenaeus vannamei*
Dominant plant species	*Phragmites australis*	*Typha angustifolia* (FWS) *Phragmites australis* (SF)	*Typha angustifolia* (FWS) *Phragmites australis* (SF)	Floating macrophytes (FM) Emergent macrophytes (SF)*	Floating macrophytes (FM) Emergent macrophytes (SF)*	Floating macrophytes (FM) Emergent macrophytes (SF)*	*Typha latifolia*
Salinity ‰		3	3	<5	<5	<5	3-15
Concentration reduction (%)							
BOD	24	37	54	40	32	29	
COD				13	20	24	
TSS	71	66	55	61	72	59	65
TP				13	2	18	31
P-PO_4	5.4	-7.6	-4				
TN							
TIN	57	66	64				
N-NO_2	90	94	83	<0.01	-59	36	
N-NO_3	68	-5.4	-2.4	16	8	42	
N-NH_4							
Chl-a	88			58	62	73	

Table 3 Continued

Reference	Schulz et al. (2003)	Schulz et al. (2003)	Schulz et al. (2003)	Sansanayuth et al. (1996)	Sansanayuth et al. (1996)	Sansanayuth et al. (1996)	Lin et al. (2002a,b)
Wetland type	HSSF	HSSF	HSSF	HSSF	HSSF	HSSF	RAS FWS-SF
A_w:A_t	0.56	0.56	0.56	unknown	unknown	unknown	0.005
HLR (m day^{-1})	5.143	3.086	1.029	0.077	0.038	0.026	0.023
HRT (day)	0.06	0.10	0.32	1.04	2.08	3.12	6.5 (FWS) 3.5 (SF)
Aquaculture species	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	shrimp	shrimp	shrimp	*Chanos chanos*
Dominant plant species	*Phragmites australis*	*Phragmites australis*	*Phragmites australis*	*Acrostichum aureum*	*Acrostichum aureum*	*Acrostichum aureum*	*Ipomoea aquatica* + *Paspalum vaginatum* (FWS) *Phragmites australis* (SF)
Salinity ‰							5
Concentration reduction (%)							
BOD				93	96	94	
COD	68	69	69				25
TSS	96	97	97	82	83	88	86
TP	68	56	58	38	68	57	
$P-PO_4$							71
TN	41	27	23	50	6	57	
TIN							95
$N-NO_2$							99
$N-NO_3$	-39	-83	-93				82
$N-NH_4$	82	83	84				98
Chl-a							76

Table 3 Continued

Reference	Lin et al. (2002a,b)	Lin et al. (2002a,b)	Lin et al. (2002a,b)
Wetland type	RAS FWS-SF	RAS FWS-SF	RAS FWS-SF
A_w:A_t	0.005	0.005	0.005
HLR (m day^{-1})	0.034	0.068	0.135
HRT (day)	4.4 (FWS) 2.4 (SF)	2.2 (FWS) 1.2 (SF)	1.1 (FWS) 0.6 (SF)
Aquaculture species	*Chanos chanos*	*Chanos chanos*	*Chanos chanos*
Dominant plant species	*Ipomoea aquatica* + *Paspalum vaginatum* (FWS) *Phragmites australis* (SF)	*Ipomoea aquatica* + *Paspalum vaginatum* (FWS) *Phragmites australis* (SF)	*Ipomoea aquatica* + *Paspalum vaginatum* (FWS) *Phragmites australis* (SF)
Salinity ‰	5	5	5
Concentration reduction (%)			
BOD			
COD	51	30	55
TSS	86	63	48
TP			
P-PO_4	45	39	32
TN			
TIN	97	98	95
N-NO_2	100	99	99
N-NO_3	99	100	97
N-NH_4	94	95	86
Chl-a	95	86	94

* The floating macrophytes consisted of *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce). Emergent macrophytes included *Typha angustifolia* (cattail), *Phragmites australis* (common reed), *Canna generalis* (canna) and *Cyperus alternifolius* (cyperus).

3.2 Suitable species of vascular plants and macro-algae

Several factors determine the suitability of plant species for use in constructed wetlands for treating brackish to saline wastewater. Besides tolerance to saline conditions, important characteristics are tolerance to high loads of wastewater, biomass production, tolerance to flooding, rooting depth and gas transport mechanisms (e.g. aerenchyma) (Brix, 1994). Plant species occurring in salt marshes are the first to be considered. However, certain plant species frequently used in constructed freshwater wetlands may also be suitable, since they can occur in brackish environments as well. Furthermore, besides vascular plant species, algae can be considered suitable for saline wastewater treatment. This chapter discusses vascular plant species and a few macro-algae that are suitable candidates for brackish to saline wastewater purification. In order to rule out possible invasive behaviour in the surroundings of the constructed wetland, a prerequisite for all species to be considered is that they be indigenous to the Netherlands.

3.2.1 Vascular plant species

Typha species

Both *Typha latifolia* L. and *T. angustifolia* L. (Figure 7a and 7b) are perennial herbaceous plants with rhizomes and belong to the group of helophytes. Both species are quite common in the Netherlands. *T. latifolia* has broader leaves and flower stalks than *T. angustifolia* (Van der Meijden, 1996). The former species is a pioneer of marshy, muddy soils that are intermittently slightly dehydrated. *T. latifolia* can grow in calm, stagnant, fresh to mesohaline water (5-18‰) to a maximum depth of 1 m (Van Ooststroom et al., 1964). The plant has no preference for a certain soil type. It can grow in both inorganic (e.g. sludge) and organic substrate, though the substrate must not be too compact (Marijnissen, 2000). *T. latifolia* prefers habitats where sedimentation occurs and organic material oxidises quickly (Van Ooststroom et al., 1964). The species grows along watersides in eutrophic environments and in nutrient rich, low pH fens and pools. The plant grows to about 1-2.5 m in height and cannot withstand high wave action. *T. latifolia* is usually found in shallower water than *T. angustifolia* (Van Ooststroom et al., 1964). It has a preference for sunny locations, but tolerates slight shadow (Marijnissen, 2000).

T. angustifolia occurs along watersides, especially of large open waters, on floating rafts in peatland areas and in reed marshes (Van der Meijden, 1996). The species grows on wet, quite nutrient rich soils or in nutrient rich water. *T. angustifolia* grows in fresh as well as mesohaline water in both sheltered places and locations exposed to current, wind and wave action (Van Ooststroom et al., 1964; Marijnissen, 2000). In the past, parts of the plant were used for several practical purposes. The felt-like cobs were used to clean lamps and chimneys, the fluff was harvested to fill pillows and beds, and the leaves were used as litter in stables (Marijnissen, 2000).

Figure 7
a) Typha latifolia, b) T. angustifolia, c) Phragmites australis

Typha species are a common feature in wetlands constructed for wastewater treatment. In vegetated wetlands, the plant produces large volumes of biomass as a consequence of its high nutrient uptake (Ciria et al., 2005). The study of Maddison et al. (2009) shows that *T. latifolia* and *Phragmites australis* have a large temporal and spatial variation in productivity. Maddison et al. (2009) found average aboveground biomass of *T. latifolia* varying from 370-1760 g dry weight m^{-2} in autumn and from 330-1380 g dry weight m^{-2} in winter (see Table 4). Harvested *Typha* material can substitute conventional fossil fuels and be used, for instance, as fuel in domestic boilers for heating (Ciria et al., 2005). Furthermore, the raw plant material can be used for insulation of houses (Maddison et al., 2009).

Phragmites australis (Cavanilles) Steudel

P. australis is a large (1-3 m) perennial grass (Figure 7c) that is very common in the Netherlands. This helophyte grows in nutrient rich water and in wet, fresh to brackish, eutrophic soils at watersides, in peat swamps, in humid forest and fields, in high salt marshes and also in dunes (Weevers et al., 1951; Van der Meijden, 1996). The species can persist in both highly acid and alkaline water. The rhizomes of *P. australis* root to a depth of more than 60 cm (Chambers et al., 2007). The species has high demands for germination and establishment. *P. australis* prefers sandy substrates and can proliferate quickly by means of aboveground and belowground rhizomes (Marijnissen, 2000).

The dispersion and growth of *P. australis* populations is most extensive under brackish conditions (salinity up to 18‰) (Ravit et al., 2007; Chambers et al., 2007). Optimal growth is achieved at 0-5‰ with growth becoming less vigorous with increasing salinity. At a salinity higher than 35‰, *P. australis* dies. The salt tolerance of *P. australis* appears to increase with developmental stage (Chambers et al., 2007). Some variants of *P. australis* are salt tolerant: *P. australis* var. *salina* occurs on strongly saline sediments in Zealand Flanders ('Zeeuws-Vlaanderen') and on the West Frisian Island of Terschelling (Weeda et al., 1994; Weevers et al., 1951). At salinities of 20-30‰ nitrogen uptake by the species is significantly lower than at salinities of 0-10‰ (Chambers et al., 1998). Sulphide can also limit establishment, growth and dispersion. In combination with high salinity, sulphide can cause stunted growth (Chambers et al., 2007). Maddison et al. (2009) found average aboveground biomass of *P. australis* varying from 610-1320 g dry weight m^{-2} in autumn and from 610-1020 g dry weight m^{-2} in winter. Vymazal and Kröpfelová (2005) reported aboveground biomass values of *P. australis* growing in constructed wetlands varying from 1652-5070 g m^{-2} (see Table 4).
The plant can be used for bank protection. Harvested plant parts serve roofing and plaiting purposes and can be used as insulation material (Maddison et al., 2009).

Schoenoplectus tabernaemontani (C.C. Gmel.) Palla

S. tabernaemontani is a perennial helophyte in the sedge family (Figure 8a). The plant produces dense stands made up of many narrow erect stems reaching 0.50-2.8 m in height (Van der Meijden, 1996).
S. tabernaemontani appears along watersides, in young dune ponds and in reed marshes. Svengsouk and Mitsch (2001) report aboveground biomass values of 130-400 g m^{-2}. *S. tabernaemontani* is used for bank protection, and the stem is plaited for use in, for instance, seats and mats (Marijnissen, 2000).

Figure 8
a) Schoenoplectus tabernaemontani, b) Bolboschoenus maritimus, c) Spartina anglica

Bolboschoenus maritimus (L.) Palla

The perennial helophyte *B. maritimus* grows on wet, fresh to rather saline soils that are rich in nutrients (Figure 8b). The plant occurs along watersides and in shallow ditches and salt marshes. *B. maritimus* reaches 0.15-1.5 m in height (Van der Meijden, 1996; Marijnissen, 2000). De Leeuw et al. (1996) reported maximum aboveground biomass of 1179 g m^{-2} (Table 4).

Spartina anglica C.E. Hubb.

S. anglica grows in the tidal zone in saline and brackish substrate rich in sludge (Figure 8c). This perennial plant forms creeping, fleshy rhizomes and upright stems (Weeda et al., 1994) reaching 0.20-1.30 m in height (Van der Meijden, 1996). The plant forms a dense and high vegetation layer (Weeda et al., 2003). Like *Phragmites australis*, *S. anglica* develops aerenchyma, and the species is able to transport considerable amounts of oxygen to the plant roots (Maricle and Lee, 2001). Architectonically, *S. anglica* resembles *P. australis* (Weeda et al., 2003). During the growing season, *S. anglica* can endure longer wet periods and deeper inundation than other wetland plants on salty substrates. Mean inundation depth can be up to 1 m with a maximum inundation period of 6 hours (Weeda et al., 2003). *S. anglica* has a high production of litter (Weeda et al., 2003). Groenendijk (1984) estimated the maximum aboveground biomass production to be 1162 g m^{-2}. The species is very efficient in the trapping of sludge (Weeda et al., 2003). *S. anglica* is planted in the tidal zone to encourage silting and prevent erosion (Marijnissen, 2000).

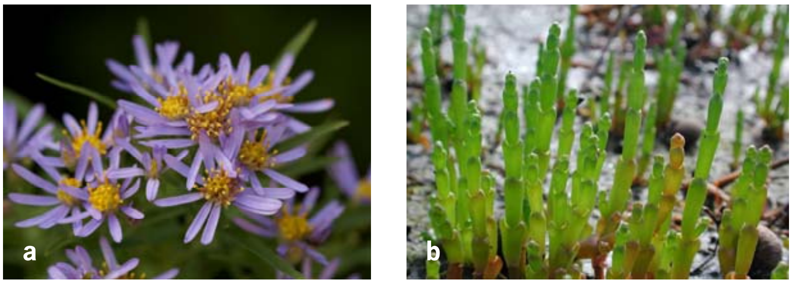

Figure 9

a) Aster tripolium, b) Salicornia europaea

Table 4

Overview of a number of plant and macro-algae characteristics of the species reported suitable for use in constructed wetlands for treating brackish to saline wastewater. Number of (*) increases with the degree of salt tolerance

Species	Height (m)	Tolerance Salinity	Inundation	Production Aboveground Biomass (g m^{-2})
Typha spp.	1-3	*	Occasional	330-1760
Phragmites australis	1-3	*	Permanent	610-5070
Schoenoplectus tabernaemontani	0.50-2.80	**	Permanent	130-400
Bolboschoenus maritimus	0.15-1.50	***	Permanent	1179
Spartina anglica	0.20-1.30	****	Occasional	1162
Aster tripolium	0.05-0.90	****	Occasional	1000-1200
Salicornia spp.	0.02-0.40	****	Occasional	876-1800

Aster tripolium L.

The annual herbaceous plant *A. tripolium* prefers salty places. It grows in wet, nutrient rich, brackish to salty substrate along the coast, but can also occur in freshwater areas like reed marshes, holms and along watersides (Figure 9a). The plant can reach 0.05-0.90 m in height (Van der Meijden, 1996). *A. tripolium* and *Salicornia* spp. are short-living, highly nitrophilous plants that can become established in organic material washed ashore and consisting mostly of algae (Weeda et al., 2003). Apart from the fact that the fleshy leaves of *A. tripolium* are a delicacy (called 'lamsoren' in the Dutch province of Zeeland), in wetlands the plant is an important food source for many animals, including geese and sheep (Weeda et al., 2003). *A. tripolium* is very productive (Table 4). When cultivated, it can be cut several times with regrowth of young shoots every 3-4 weeks (internet site 3).

***Salicornia* species**

In the Netherlands and Belgium, three species of *Salicornia* occur: *S. europaea* L. (Figure 9b), *S. procumbens Smith* and *S. pusilla Woods*. The former two species are common in the Netherlands, whereas the latter is rare. *Salicornia* species are annual herbs and belong to the group of obligatory halophytes. Although *Salicornia* can tolerate freshwater to some extent, NaCl is required for normal growth (Ayala and O'Leary, 1995). The plants (0.02-0.40 m) occur mainly in tidal areas, but also grow in inland locations. The plants grow in open, strongly saline and wet conditions. *S. procumbens* occurs in places below mean high water levels, *S. europaea* occurs above mean high water levels (Van der Meijden, 1996; Davy et al., 2001). The root system tends to be superficial, often penetrating less than 10-20 cm into the sediment. Larger plants develop several main roots that are highly branched and woody (Davy et al., 2001). *Salicornia* species can transport oxygen via aerenchyma to the root system (Davy et al., 2001). As such, they can grow in places where the top layer of the substrate (1 cm) is anaerobic (Weeda et al., 2003). *Salicornia* species in intertidal as well as inland saline areas can grow in a diverse range of marine sediments, ranging from gravels and sands mixed with shells to silts and fine clays. The densest stands of *Salicornia* seem to be found on silts and clays (Davy et al., 2001).

Salicornia species are extremely tolerant to regular flooding. However, growth of *S. europaea* appears to be slower under continuously waterlogged conditions compared to free drainage conditions. Optimal growth generally occurs at salinities equal to or less than 17‰. Species that are characteristic of low salt marshes (like *S. europaea*) seem to have higher growth rates than species that occur at higher elevations (Davy et al., 2001). The net annual productivity (aboveground biomass) of *Salicornia* species in the salt marshes of the north Norfolk coast is estimated at 876 g m^{-2} yr^{-1}. Growth and, especially, seed production can be significantly increased within a single growing season by the regular addition of N-NO_3^- and N-NH_4^+. *S. europaea* is able to accumulate nitrogen with increasing nitrate availability (Davy et al., 2001).

Germination, which takes place in the spring, is at low temperatures and is even possible in substrates with salinities almost equal to that of seawater (Weeda et al., 2003). However, germination seems to be best under less saline conditions (Marijnissen, 2000). Saplings are susceptible to dehydration of the substrate (Weeda et al., 2003). *S. procumbens* is important in trapping sludge (Marijnissen, 2000). In autumn, the plants change colour from fresh green to orange/red (Marijnissen, 2000).

Salicornia is edible and has long been consumed as a vegetable. In arid regions, *S. bigelovii* seems very promising for use in seawater-irrigated agriculture. The species has been grown as an oilseed and forage crop. The seeds contain high levels of oil (26-33%) and protein (31%), similar to soybeans and other oilseed crops (Glenn et al., 1991 and 1999). *Salicornia* can make up part of the diets fed to sheep and goats, and *Salicornia* meal, which is a by-product of oil extraction, can replace conventional seed meals in livestock diets (Davy et al., 2001). In Mexico, field trials were conducted over a six-year period in which effluent originating from shrimp aquaculture was used to irrigate *S. bigelovii*. The species produced a mean annual total biomass of 1800 g m^{-2} and 200 g m^{-2} of oilseed (Glenn et al., 1999). These yields of seed and biomass equal or exceed conventional freshwater oilseed crops (Glenn et al., 1991).

3.2.2 Algae

Algae belong to a diverse group of typically autotrophic organisms. They differ in degree of complexity, ranging from unicellular to multicellular forms. Algae can occur in freshwater habitats as well as in marine environments both in floating form and attached to substrate such as rocks and other algae (Raven et al., 1999). Algae are highly productive. In freshwater habitats, they can be the primary contributor to the productivity of these systems (Raven et al., 1999). A distinction can be made between micro-algae (approximately 1-50 μm) and macro-algae (seaweed or kelp).

Macro-algae are produced for food and for their content of mucilaginous substances (agar). They are cultivated either directly in the sea or, in some cases, as small individual plants, kept in suspension in agitated ponds (internet site 4). Most described micro-algae species produce unique substances, such as fatty acids, antioxidants, polymers and carotenoids. They therefore seem promising for use in producing primary products that can be applied, for instance, in the fish-feed industry, as nutrition supplements, in bioplastics and in food pigments. Micro-algae are generally cultivated in photobioreactors, though outdoor ponds are also used (Figure 10). According to R. Wijffels (bioprocess technology, Wageningen UR), the cultivation of algae can become cost-effective if multiple product types are extracted from the algal biomass (Wolkers, 2010). Algae can also be deployed to convert nutrients in wastewater to biomass and are an interesting option for manufacture of biodiesel. However, the production of biodiesel with algae is most efficient in large-scale systems which are not yet competitive with traditional diesel production from crude mineral oil (internet site 5). Today, several relatively small companies cultivate micro-algae in the Netherlands (Ingrepro, LGem, Aquaphyto). However, there remains a lack of in-depth knowledge about cost-effective, large-scale production. To remedy this gap Wageningen UR is currently building a facility for research on sustainable and economically viable micro-algae cultivation systems. The facility is called AlgaePARC (Algae Production and Research Centre, see internet site 6).

Ulva lactuca L.

The bloom-forming green alga *Ulva lactuca* (sea lettuce) is common along temperate seashores throughout the world and is often an abundant species in estuaries (Lartigue et al., 2003). The species is also common in the tidal areas around the North Sea (internet site 7). *U. lactuca* (Figure 11a) is found at all levels of the intertidal zone. In more northerly latitudes and in brackish habitats, the species also occurs in the shallow sublittoral zone (internet site 8). The algae have a high survival rate in low saline waters (Tsagkamilis et al., 2010). *U. lactuca* has a thin, flat thallus, two cell layers thick, that may reach 1 m or more in length and up to 30 cm across (Raven et al., 1999, internet site 8). The thallus is anchored to the substrate by a small disc-shaped holdfast, but it can be detached from the substrate in very sheltered conditions, forming extensive floating communities that can still grow (internet site 8). *U. lactuca* dies back intermittently (Fujita 1985). Accumulation and decay of algal biomass can lead to release of methane, the poisonous hydrogen sulphide, and other gasses.

U. lactuca grows quickly due to its high photosynthetic capacity and relatively rapid nutrient uptake compared to other macro-algae (Lartigue et al., 2003; Ale et al., 2010). Like a number of bloom-forming algae, *U. lactuca* can take up nitrogen in the form of both NH_4^+ and NO_3^- at similar rates, whereas other species have higher affinity for NH_4^+ (Teichberg et al., 2008). Despite *U. lactuca*'s high nutrient uptake rates, it has a limited capacity to store inorganic nutrients (Fujita, 1985). According to Fujita (1985), the uptake of NH_4^+ by *U. lactuca* at high nitrogen flux is much lower than uptake by the species grown at low nitrogen flux or after a period of nitrogen starvation. The same study found similar responses for two other algae species. The effect of (long-term) high or low nutrient conditions on macro-algae may, therefore, play a role in their responses to elevated nutrient conditions (Teichberg et al., 2008).

Lartigue et al. (2003) studied the NH_4^+ and NO_3^- uptake by *U. lactuca* in three different salinity regimes: 20‰, 25‰ and 30‰. The species was collected from sites with ambient salinity levels of about 25‰. In the experiment, initial NH_4^+ and NO_3^- concentrations were both 30 μM. Uptake rates of NH_4^+ and NO_3^- were, respectively, between 6.8 and 12.4 μmol g^{-1} fresh weight h^{-1} and between 3.7 and 5.6 μmol g^{-1} fresh weight h^{-1} (Table 5).

Figure 10
Outdoor pond to cultivate algae

Table 5
Ammonium and nitrate uptake rates by Ulva lactuca at different salinities

Salinity (‰)	Uptake rate (µmol g^{-1} FWh^{-1})	Uptake rate (µmol g^{-1} DW h^{-1})*
NH_4^+ uptake		
20	12.36 ± 1.97	51.50 ± 8.2
25	6.81 ± 0.62	28.38 ± 2.5
30	7.18 ± 1.53	29.92 ± 6.4
NO_3^- uptake		
20	5.63 ± 0.83	23.46 ± 3.5
25	5.39 ± 0.45	22.46 ± 1.9
30	3.69 ± 0.54	15.38 ± 2.3

* A conversion factor FW/DW of 24 is used to calculate uptake rates per *U. lactuca* dry weight.

Tsagkamilis et al. (2009) found PO_4^{3-} uptake rates by *U. lactuca* of 2.13 µmol PO_4^{3-} g^{-1} dry weight h^{-1} which is equivalent to 0.1 g PO_4^{3-} m^{-2} h^{-1}. This amount of phosphate was absorbed by an algal biomass of approximately 495 g^{-1} dry weight m^{-2}. Msuya and Neori (2010) reported specific growth rates for *U. lactuca* of 12-17% fresh weight day^{-1} and fresh algal biomass yields of 158 to 283 g m^{-2} day^{-1}.

U. lactuca has various uses. The macro-algae contains a number of important vitamins and trace elements and can be eaten as a vegetable (Raven et al., 1999). The species is also used as a seaweed biofilter for marine culture effluents (Neori et al., 1991).

Nowadays, research is being done on energy production using marine macro-algae. At Aarhus University in Denmark, a project by the National Environmental Research Institute of Denmark (NERI) aims to develop a method for producing liquid, gaseous and solid biofuel from *U. lactuca*.

Laminaria species

The genus *Laminaria* belongs to the group of the brown algae of which the larger algae are called kelps (Raven et al., 1999). Two species of the *Laminaria* genus occurring in the Netherlands are *Laminaria saccharina* and *L. digitata* (Figure 11b and 11c). The first species occurs in the Eastern Scheldt ('Oosterschelde') and the Wadden Sea, whereas the latter species grows in marine habitats near Texel, Den Helder, Westkapelle and Neeltje Jans and is characteristic for the submerged (artificial) rock shores (internet site 7). *L. digitata* will not grow in sheltered habitats (Seip, 1980). *L. saccharina* grows attached to submerged rocks in sheltered low-tidal areas and in deep pools. The species is often found together with *L. digitata*. *L. saccharina* has a relatively short stem with an unbranched leaf attached. The edge of the filamentous leaves are rippled (see Figure 11b). The thallus of *L. digitata* is branched and the leaves are generally smooth (internet site 9). Both *L. saccharina* and *L. digitata* can reach lengths of up to 4 m (internet site 7). Seip (1980) mentions a growth rate of 5.5% fresh weight day^{-1} for *L. digitata*. *Laminaria* species grow slower than *Ulva* species (Pedersen et al., 2010).

L. saccharina produces a useful substance called mannitol which is utilised in all kinds of foods as a sweetener, an anti-caking agent and as an additive to maintain moisture in food. *L. digitata* is edible (internet site 7) and in Ireland and France is harvested in small quantities as a vegetable. Furthermore, it is harvested in France (Brittany) for alginate production (internet site 10). In many northern regions, kelp is locally harvested for immediate use as a fertiliser. Kelp is also harvested for its ash, which is rich in sodium and potassium salts (Raven et al., 1999).

The application of macro-algae in purification of aquaculture effluent is being studied in a Dutch project called 'Vis, schelp en wier'. This cooperative effort between Hortimare, Seafarm, Grovisco, IMARES and Plant Research International (both part of Wageningen UR) started in August 2009 (internet site 11).

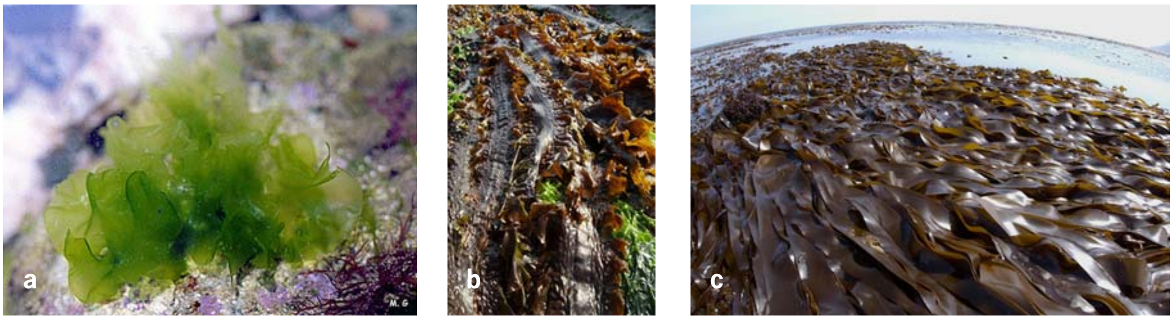

Figure 11
a) Ulva lactuca, b) Laminaria saccharina, c) Laminaria digitata

3.3 Suitable species integrated in brackish to saline wetland unit designs

This section discusses four wetland designs for treating brackish to saline effluent originating from aquaculture. The designs are shown in Figures 12a-12d, drawn for illustrative purposes only and not to scale. All designs consist of an aquaculture pond system linked to a wetland unit composed of several compartments arranged in series. Recirculating wetland systems are not considered. The treated effluent is collected at the end of the wetland unit, to enable the quality of the effluent to be evaluated before it is drained to receiving waters.

In all four wetland designs, a pre-settling basin is placed before the wetland to trap the quickest settling suspended solids. A mechanic filter can be used between the settling basin and wetland to enhance filtering of suspended particles. Since an estimated 70% of the N-NH_4^+ present in aquaculture effluent is associated with organic solids and 47-84% of P-PO_4^{3-} is particle-bound, a settling basin is a very effective method to remove a large part of both pollutants.

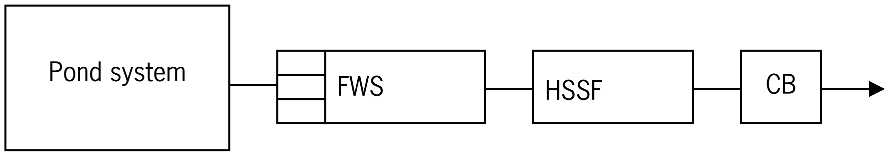

Figure 12a

Wetland design with pre-settling basin, surface-flow wetland (FWS), horizontal subsurface-flow wetland (HSSF) and collection basin (CB)

The first wetland design consists of a surface-flow wetland (FWS) situated before a horizontal subsurface-flow (HSSF) wetland (Figure 12a). In these wetlands the removal of nitrogen is promoted by plant uptake and by nitrification and denitrification processes. Phosphates can be taken up by plants and adsorb to and precipitate with iron, aluminium, calcium and soil particles in the sediment of both wetland types. To ensure continuous flow, the FWS can be elevated above the HSSF. Nitrification processes in the FWS wetland are assumed to be sufficient to ensure that wastewater rich in nitrate enters the HSSF wetland. Due to denitrification processes, part of the nitrogen initially present in the wastewater is withdrawn from the system. The FWS compartment (0.2-0.4 m deep) is planted with *Spartina anglica* or a combination of salt-tolerant species. Salt marsh species most commonly found with *S. anglica* are *Aster tripolium*, *Salicornia europaea*, *Suaeda maritima*, *Atriplex portulacoides* and *Puccinellia maritima*. In brackish and freshwater habitats, *S. anglica* can occur alongside other plant species, such as *Bolboschoenus maritimus* and *Phragmites australis* (Cope and Gray 2009). Several studies reported in Table 3 mention *Typha* species as the dominant plant in the FWS compartment. Salinity values, however, were 3-15‰. At higher salinity, the performance of *Typha* species in the FWS wetland and thus removal efficiency is probably suboptimal. Due to its rooting system characteristics, *Phragmites australis* seems suitable for HSSF wetlands. Addition of rainwater could reduce the salinity of the wastewater.

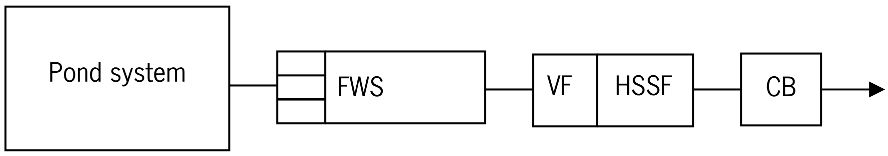

Figure 12b

Wetland design with pre-settling basin, surface-flow wetland (FWS), a hybrid wetland (VF + HSSF) and collection basin (CB)

The first wetland design can be supplemented with a vertical flow (VF) wetland situated before the HSSF wetland (Figure 12b). The assumption made here is that aerobic microbial populations and pollutant removal mechanisms are not stimulated enough in the FWS wetland, resulting in limited nitrification and thus in FWS effluents low in nitrate. Since VF wetlands are not permanently water saturated, the filter bed is better aerated compared to HSSF systems. Denitrification processes are still stimulated in the HSSF wetland. Like the HSSF wetland, the VF system can be planted with *Phragmites australis*.

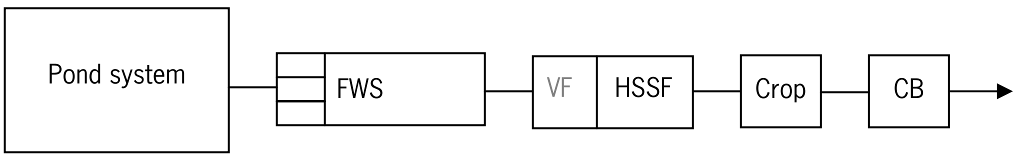

Figure 12c

Wetland design with a pre-settling basin, a surface-flow wetland (FWS), either a HSSF or a hybrid wetland, a crop field and a collection basin (CB)

The third option is to supplement the first two wetland designs (i.e. Figure 12a and 12b) with a saline crop field after the subsurface wetland(s) (Figure 12c). The field can be regularly flooded with effluent originating from the wetlands. *Salicornia* species and *Aster tripolium* can be considered as suitable crops since the species are tolerant to regular flooding and can be used for human consumption as well as to provide food for livestock and wildlife.

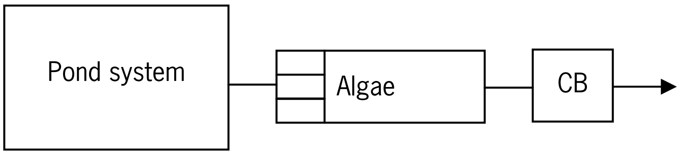

Figure 12d
Wetland design with a pre-settling basin, a basin with algae and a collection basin (CB)

The last wetland design consists of a pre-settling basin and a compartment with macro-algae (e.g. *Ulva lactuca*, Figure 12d). A large part of the suspended solids, nitrogen and phosphorus present in the aquaculture effluent is removed in the settling compartment. In the basin with macro-algae, more sedimentation can occur and the algae can take up nitrogen species and phosphates very efficiently. Especially *U. lactuca* shows rapid growth under these conditions. Regular harvest of the produced biomass is important to prevent decease of the algae material and its additional effects (e.g. decrease in oxygen level and release of nutrients). In outdoor situations where effluent is treated by wetlands with only macro-algae it is important to keep in mind that in periods without algae biomass, contaminant removal will probably be minimal.

3.4 Example wetland estimation

This section presents an example estimation of the area of constructed wetland required for a given target efficiency of removal of suspended solids from shrimp aquaculture effluent. The system consists of eight ponds, each with the dimensions 50.0 x 2.0 x 0.7 m (L x W x D) and a water depth of 0.5 m. The unit used to treat the effluent is composed of a FWS wetland in series with a HSSF system. Equation 1 of Box 1 is used to estimate the required area. Table 6 shows the parameter values in this example. Several assumptions are made:

- Fishponds have a regular daily effluent of approximately 5% of the pond volume (Lin et al., 2002b).
- The FWS wetland reduces suspended solids by 30%, based on results in Lin et al. (2002a).
- There is a continuous flow of wastewater through the wetland system.
- The background concentration of suspended solids is not taken into account.
- The effluent target concentration of suspended solids of the HSSF wetland is 5 mg l^{-1}.

With the parameter values mentioned, estimated required FWS and HSSF wetland areas are about 15 and 7 m^2. This results in an $A_{wetland}$: A_{pond} ratio of 0.03 (22/800). In this example the HRT and HLR are 0.06 days and 1.4 m day^{-1} and 0.09 days and 3 m day^{-1} for the FWS and HSSF wetland, respectively. The removal of suspended solids can be improved by placing a settling basin before the wetland unit.

Table 6
Characteristics of wetland types. For the description of the parameters, see Box 1

Parameter	Wetland type		Wetland type	
	FWS	Source	HSSF	Source
Q (m^3 day^{-1})	20	-	20	-
C_i(mg l^{-1})	88	Sansanayuth et al. (1996)	61.6	-
C_e(mg l^{-1})	61.6	-	5	-
k_{ss}(day^{-1})	6	Lin et al. (2005)	6	Lin et al. (2005)
ε (-)	0.4	Sansanayuth et al. (1996)	0.45	Lin et al. (2005)
h_w(m)	0.2	Kadlec and Wallace (2009)	0.6	Vymazal (2005)

4 Conclusions and recommendations

This report has looked at the use of constructed wetlands for treatment of traditional as well as slightly saline wastewater. Constructed wetlands have proven to be successful in removing a diverse array of pollutants originating from different wastewater sources. Most knowledge of and experience with constructed wetlands is derived from freshwater treatment wetlands. Nonetheless, there is a growing body of literature on contaminant removal from mesohaline (5-18‰) aquaculture effluent. Constructed wetlands are an ecologically attractive and economical method for treatment of aquaculture wastewater.

Contaminants present in wastewater are removed or reduced by a range of physical, chemical and biological processes. The efficiency of contaminant removal depends, amongst other things, on wastewater constituents, load concentration, constructed wetland type, hydraulic loading rate (HLR) and hydraulic retention time (HRT). Constructed wetland types can be linked in series to combine the advantages of each type, resulting in a higher pollutant removal efficiency and reduction of surface area requirement.

Constructed wetlands are, moreover, a cost-effective alternative to conventional wastewater treatment. Construction and operation costs are low. However, annual harvesting of part of the wetland vegetation is recommended, and horizontal subsurface-flow wetlands require inlet zone maintenance about once every ten years. When using pre-settling basins as a first wetland compartment to trap the quickest settling fraction of suspended material, sludge will occasionally need to be removed.

Saline aquaculture effluent contains particulates, organic matter (including algae), nitrogen and phosphorus. An estimated 70% of $N\text{-}NH_4^+$ is associated with organic solids and 47-84% of $P\text{-}PO_4^{3-}$ is particle-bound. The effluent is characterised by a relatively dilute concentration of pollutants.

Constructed wetlands for treatment of mesohaline aquaculture effluent typically consist of a surface-flow wetland linked in series to a subsurface-flow wetland. In some cases, aquaculture water is recirculated. *Phragmites australis* and *Typha* species tend to be the dominant wetland vegetation. These species are indigenous to the Netherlands and Western Europe. Mixtures of salt-tolerant vascular plant species are also used. High removal rates are reported for suspended solids and phytoplankton (48-99% and 58-95%, respectively). Reduction values for BOD_5 and COD are highly variable between studies. Nitrogen and phosphorus removal efficiencies are highly variable as well, depending on the hydraulic and nutrient loading rates.

For saline wastewater treatment in the Netherlands by means of constructed wetlands, use of indigenous species is strongly recommended in order to rule out invasive behaviour. Both plant species commonly used in constructed wetlands and species found in tidal areas show substantial biomass production. The advantage of *Phragmites australis* and *Typha* species in the treatment of wastewater over species accustomed to tidal areas is in their rooting depth. High salinity, however, can diminish the performance of these species. The macro-alga *Ulva lactuca* shows high rates of nutrient uptake and is able to grow in full-strength seawater. The species dies back intermittently. Accumulation of *U. lactuca* must be limited to prevent the release of the poisonous hydrogen sulphide, methane and other gases. Further (experimental) research is necessary to gain a better understanding of the performance of plant species at various saline levels and high wastewater loads and to determine their contribution to the removal of contaminants.

Salicornia species, *Aster tripolium* and *U. lactuca* can be used for human consumption. At the moment, several studies are conducted to develop methods for producing biofuel from *U. lactuca* and other macro-algae and for producing fodder from *Salicornia* spp., *Suaeda* spp. and *Atriplex* species. More research on economically viable ways to process and reuse sludge and plant biomass is recommended.

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Internet site 3: <www.scrops.com/Aster.html>

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Internet site 5: <www.algae.wur.nl/UK/projects/Biofuels+from+microalgae.+Scenario+studies+for+algae+plants/default.htm>

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Pictures

Cover with *Salicornia*-vegetation.
<www.flickr.com/photos/21933510@N07/3209445930/sizes/o/in/photostream/>

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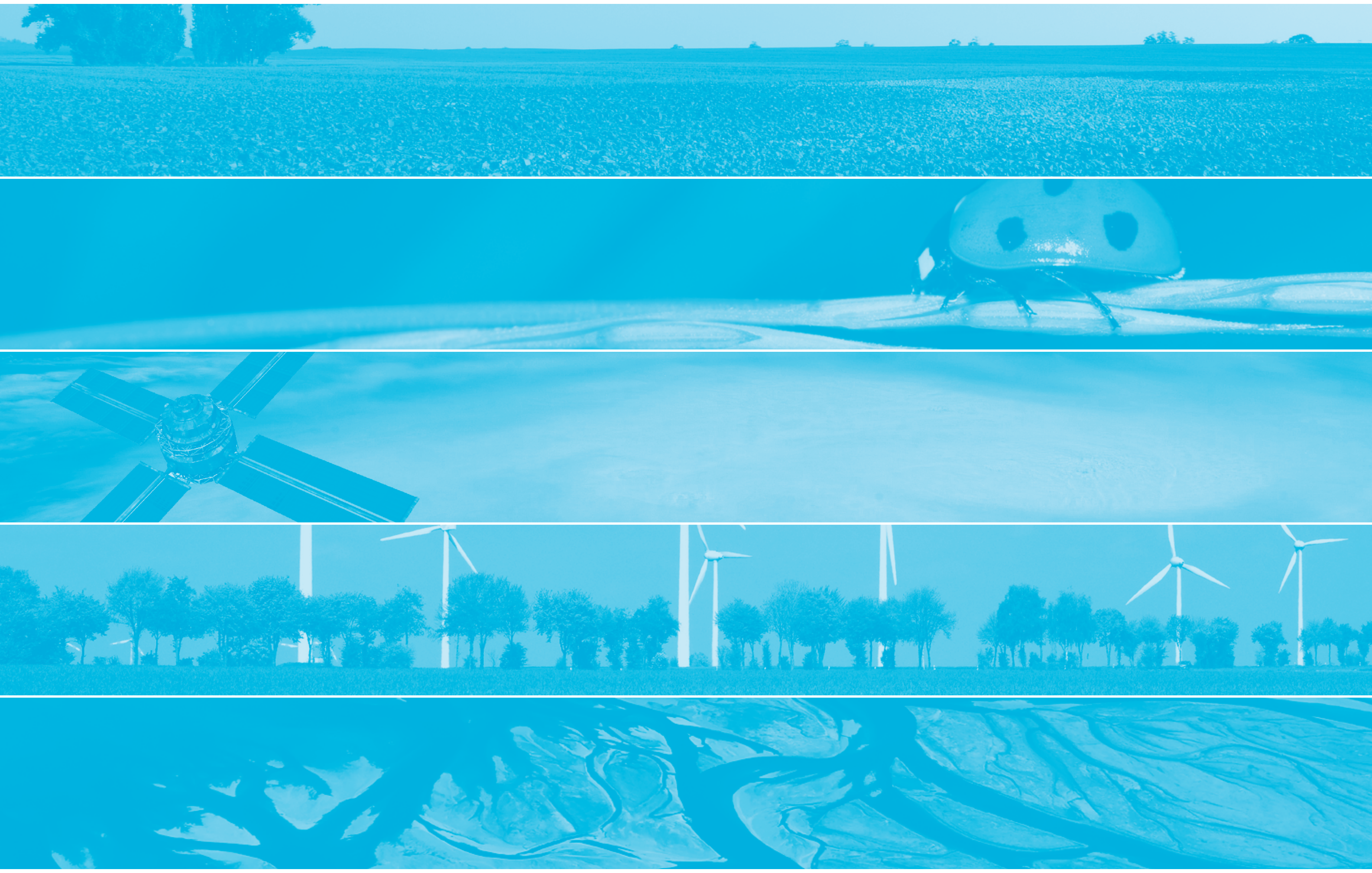
Figure 11b. <www.celtnet.org.uk/recipes/ancient/wild-food-entry.php?term=Sugar%20Kelp>
Figure 11c. <www.seaweed.ie/descriptions/Laminaria_digitata.html>



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