CHAPTER III

BIBLIOGRAPHICAL REVIEW

3.1 Introduction in wetlands

Wetlands are found in all continents, except for Antarctica and in all climates, ranging from the tropics to the frozen tundra. Any estimate of wetland extension in the world is difficult and depends on the definition of wetland, but different scientists have tried, and according to Matthews and Fung (1987) and Aselman and Crutzen (1989, 1990) the estimated area of world’s wetland extension seems to be 7 to 8 millions km$^2$.

The technical meaning of the term wetland includes a wide range of ecosystems from areas that are never flooded to areas that are deeply flooded all of the time. Areas that are not flooded may still be classified as wetlands because of saturated soil conditions where water is at or below the ground surface during part of a typical growing season. Wetland areas that are deeply flooded grade imperceptibly into aquatic ecosystems as water depth exceeds the growth limits of emergent or submergent vegetation. Figure 3.1 shows how wetlands lie on a continuum between dry lands (uplands) and deeply flooded lands (aquatic systems) (Kadlec and Knight, 1996).

![Figure 3.1](image.png)

**Figure 3.1** Wetlands are transitional areas between uplands, where excessive water is not a factor for plant growth, and aquatic ecosystems, where excessive flooding excludes rooted, emergent vegetation (Kadlec and Knight, 1996).
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The most consistent attribute of wetlands is the presence of water during some or all of an average annual period. Wetlands are areas where the soil is saturated with water or where shallow standing water results in the absence of plants species, which depend on aerobic soil conditions. Wetlands are dominated by plants species that are adapted to growing in seasonally or continuously flooded soils with resulting anaerobic conditions or low-oxygen conditions. At their upslope margin, wetlands can be distinguished from uplands by the latter’s tendency to remain flooded or saturated for less than 7 to 30 days each year, a short enough period so that oxygen and other soil conditions do not limit the plant growth. At their down gradient edge, wetlands grade into aquatic system which are flooded to a depth or at a duration where emergent, rooted plants cannot survive. The average water depth, which typically separates wetlands from adjacent aquatic ecosystems, is in the range of 1 to 2 m (Kadlec and Knight, 1996).

Typically, there are three main components used to define wetlands (Vymazal, 1998):

1. Wetlands are distinguished by presence of water.
2. Wetlands often have unique soils that differ from adjacent uplands
3. Wetlands support vegetation adapted to the wet conditions (hydrophytes), and conversely are characterized by an absence of flood-intolerant vegetation.

Figure 3. 2 shows structural components typical of wetlands ecosystems. Starting with the unaltered sediments or bedrock below the wetlands, these typical components are (IWA, 2000):

- Underlying strata: unaltered organic, mineral or lithic strata, which are typically saturated with or impervious to water and below the active rooting zone of the wetland vegetation.

- Hydric soils: the mineral to organic soil layer of the wetland, which is frequently saturated with water and contains roots, rhizomes, tubers, tunnels, burrows and other active connections to the surface environments.

- Detritus: the accumulation of live and dead organic material in a wetland, which consists of dead emergent plant material, dead algae, living and dead animals (primarily invertebrates) and microbes (fungi and bacteria).

- Water: standing water, which provides a habitat for aquatic organisms including fish and other vertebrate animals, submerged and floating species that depend on water for buoyancy and support, living algae and population of microbes.

- Emergent vegetation: vascular, rooted, hydrophytic plants species, which contain structural components that emerge above the water surface, including both herbaceous and woody plants species.
The National Research Council defines wetland as follows: a wetland is an ecosystem that depends on constant or recurrent, shallow inundation or saturation at or near the surface of the substrate. The minimum essential characteristics of a wetland are recurrent, sustained inundation or saturation at or near the surface and the presence of physical, chemical, and biological features reflective of recurrent, sustained inundation or saturation (Vymazal, 1998).

3.2 Wetlands for wastewater treatment

Natural wetlands have been used for wastewater treatment for centuries. In many cases, the reasoning behind this use was disposal, rather than treatment and the wetland simply served as a convenient recipient that was closer than the nearest river or other way. Uncontrolled discharge of wastewater led in many cases to an irreversible degradation of many wetlands areas, which generated nuisance places. However, there has been an explosive growth of knowledge about and a radical change of attitude towards wetlands during the last few decades. Wetlands have been recognised as providing many benefits, including water supply and control (recharge of groundwater aquifers, drinking water, irrigation, flood control, water quality and wastewater treatment), use of plants, wildlife, integrated system and aquaculture, erosion control, energy, education and training recreations and reclamation (Vymazal, 1998).

Natural wetlands are characterised by extreme variability in functional components, making it virtually impossible to predict responses to wastewater application and to translate results from one geographical area to another. Although significant improvement in the quality of the wastewater is generally observed as a result of flow through natural wetlands, the extent of their treatment capability is largely unknown. Constructed wetlands can be built with a much greater degree of control, thus allowing the establishment of experimental treatment facilities with a well-defined composition of substrate, type of
vegetation, and flow pattern. In addition, constructed wetland offer several additional advantages compared to natural wetlands including site selection, flexibility in sizing, and most importantly, control over the hydraulic pathways and retention time (Vymazal, 1998).

The pollutants in such systems are removed through a combination of physical, chemical, and biological processes including sedimentation, precipitation, adsorption to soil particles, assimilation by the plant tissue, and microbial transformations (Brix, 1993).

Constructed wetlands treatment systems are engineered systems that have been designed and constructed to utilise the natural processes involving wetlands vegetation, soils and their associated microbial assemblages to assist in treating wastewater. They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment. Some of these systems have been designed and operated with the sole purpose of treating wastewater, while others have been implemented with multiple-use objectives in mind, such as using treated wastewater effluent as a water source for the creation and restoration of wetland habitat for wildlife and environmental enhancement (Vymazal, 1998).

3.2.1 Types of constructed wetlands.

Based on the position of the free water layer (underneath or over the ground), constructed wetlands may be classified in the following way:

1) Free water surface treatment wetlands.
   1.1) FWS treatment wetlands with emergent macrophytes.
   1.2) FWS treatment wetlands with free-floating macrophytes.
   1.3) FWS treatment wetlands with floating-leaved, bottom-rooted macrophytes.
   1.4) FWS treatment wetlands with floating mats.
   1.5) FWS treatment wetland with submersed macrophytes.

2) Subsurface flow treatment wetlands.
   2.1) Horizontal-flow systems
   2.2) Vertical-flow systems

1) Free water surface treatment wetlands

Constructed free water surface treatment wetlands mimic the hydrological regime of natural wetlands, have some properties in common with facultative lagoons and have some important structural and functional differences. Water column processes in deeper zones within treatment wetlands are nearly identical to ponds with surface autotrophic zones dominated by planktonic or filamentous algae, or by floating or submerged aquatic macrophytes. Deeper zones tend to be dominated by anaerobic microbial processes in the absence of light. However, shallow emergent macrophyte zones in treatment wetlands and aerobic lagoons are quite different. Emergent wetland plants tend to cool and shade the water. Net carbon production in vegetated wetlands tends to be higher than that in facultative ponds because of high gross primary production in the form of structural carbon, accompanied by resistance to degradation and low rates of decomposition of
organic carbon in the oxygen-deficient water column. This high availability of carbon and the short diffusional gradients in shallow vegetated wetlands result in differences in biogeochemical cycling compared with ponds and lagoons (IWA, 2000).

During the process of elemental cycling within the wetland, chemical free energy is extracted by the heterotrophic biota, and fixed carbon and nitrogen are lost to the atmosphere. A smaller portion of the phosphorus and other non-volatile elements can be lost from the mineral cycle and buried in accreting sediments within the wetlands. Wetlands are autotrophic ecosystems, and the traditional fixed carbon and nitrogen from the atmosphere is processed simultaneously with the pollutants introduced from the wastewater source. The net effect of these complex processes is a general decrease in pollutant concentrations between the inlet and outlet of treatment wetlands. However, because of the internal autotrophic processes of wetland, outflow pollutant concentrations are seldom zero, and in some cases for some parameters, they can exceed inflow concentrations (IWA, 2000).

1.1) FWS treatment wetlands with emergent macrophytes.

A FWS wetland consists of a shallow basin constructed of soil or other medium to support the roots of vegetation, and a water control structure that maintains a shallow depth of water (see Figure 3.3). FWS wetlands look and act much like natural marshes, and they can provide wildlife habitat and aesthetic benefits as well as water treatment. In FWS wetlands, the near-surface layer is aerobic, whereas the deeper water and substrate are usually anaerobic. Typical water depths range from a few centimetres up to a metre (IWA, 2000).

FWS treatment wetlands function as land-intensive biological treatment systems. Inflow water containing particulate and dissolved pollutants slows and spreads through a large area of shallows water and emerged vegetation. Particulates, typically measured as total suspended soils (TSS), tend to settle and are trapped due to lowered flow velocities and sheltering from wind. These particulates contain BOD components, fixed forms of total nitrogen (TN) and total phosphorus (TP), and trace levels of metals and organics. These insoluble pollutants enter into the biogeochemical element cycles within the water column and surface soils of the wetlands. At the same time, a fraction of dissolved BOD, TN, TP and trace elements are sorbed by soils and active microbial and plants populations throughout the wetlands environment. These dissolved elements also enter the overall mineral cycles of the wetlands ecosystems (Kadlec and Knight, 1996).

Nitrogen is most effectively removed in FWS systems by nitrification and denitrification. Ammonia is oxidized by nitrifying bacteria in aerobic zones, and nitrate is converted to free nitrogen or nitrous oxide in the anoxic zones by denitrifying bacteria. FWS systems provide the sustainable removal of phosphorus but at relatively slow rates. Phosphorus removal in FWS systems occurs from absorption, complexation and precipitation. However, the major process in phosphorus removal (precipitation with ions of Al, Fe and Ca) is limited by little contact between column and the soil (Vymazal et al., 1998).
The plants that are most often used in FWS constructed wetlands are persistent emergent plants such as bulrushes (*Scripus* spp), spikerush (*Eleocharis* spp), sedges (*Cyperus* spp and *Carex* spp), rushes (*Juncus* spp), common reed (*Phragmites australis*), reed canarygrass (*Phalaris arundinacea*), sweet mannagrass (*Glyceria maxima*) and cattails (*Typha* spp). Not all wetlands species are suitable for wastewater treatment because plants in treatment wetlands must be able to tolerate the combination of continuous flooding and exposure to wastewater or stormwater containing relatively high and often variable concentrations of pollutants (IWA, 2000).

1.2) FWS treatment wetlands with free-floating macrophytes

Floating aquatic plant (FAP) treatment system consist of one or more shallow ponds which one or more species of water-tolerant, floating vascular plants are grown (see Figure 3. 4). The shallower depths and the presence of aquatic macrophytes in place of algae are the major difference between aquatic treatment systems and stabilization ponds. The presence of plants is of great practical significance because the effluent from aquatic systems is of higher quality than the effluent from stabilization pond systems for equivalent or shorter detention times (IWA, 2000).

In FAP systems used for municipal wastewater, the carbonaceous BOD (cBOD) and TSS are removed principally by bacterial metabolism and physical sedimentation. In systems used to treat cBOD and TSS, the plants themselves bring about very little actual treatment of the wastewater. Their function is to provide components of the aquatic that improve the wastewater treatment capability and/or the reliability of that environment. In aquatic treatment systems designed to remove nutrients (N and P), plants uptake can contribute to the removals, especially where plants are harvested frequently (IWA, 2000).
The principal floating plant species used in FAP treatment systems are water hyacinth (Eichhornia crassipes) and duckweed (Lemna spp). These and other floating species, such as water lettuce (Pistia stratiotes) and mosquito ferns (Azolla spp.) can occur in any FWS wetland (IWA, 2000).

The use of water hyacinths has been limited in geographic location to warm-weather regions because of the sensitivity of water hyacinth to freezing conditions (IWA, 2000). This plant has been one of the most studied aquatic plants because of its detrimental as well as beneficial effects on the environment. Hyacinth systems can be designed for treatment of raw wastewater, primary effluent, upgrading of existing secondary treatment systems, or for advanced secondary or even tertiary treatment. The role of water hyacinth in the process of suspended solids is well documented. The removal of suspended solids occurs through entrapment in the plant root zone by gravity sedimentation in the quiescent water beneath the surface mat of hyacinth plants. The dense cover of water hyacinth effectively reduces the effect of wind mixing and also minimises thermal mixing. Furthermore, electrical charges associated with hyacinths roots are reported to react with opposite charges on colloidal particles such as suspended solids, causing them to adhere to plant roots, where they are removed from the wastewater stream and slowly digested and assimilated by the plant and microorganisms. The efficiency of water hyacinth in removing BOD and in providing good conditions for microbial nitrification is related to their capability of transporting oxygen from the foliage to the rhizosphere. The extensive root system of the water hyacinth provides a huge surface area for attached microorganisms, thus increasing the potential for decomposition of organic matter (Vymazal et al., 1998).

Plant uptake, ammonia volatilization, and nitrification-denitrification all contribute to nitrogen removal in hyacinth systems. The major factor responsible for this additional removal is believed to be nitrification-denitrification. The microorganisms bugs can flourish attached to the hyacinth roots, which provide oxygen, while adjacent microsites and benthic layer will provide the anaerobic conditions and the carbon source for denitrification. Otherwise, the only significant removal pathway for phosphorus removal in water hyacinth-based treatment systems is plant uptake and that will usually not exceed 30 to 50% of phosphorus present in typical municipal wastewater. However, the removal will not even approach that range without a careful vegetation, management programme involving, frequent harvesting (Vymazal et al., 1998).

Duckweed systems have been developed in colder climates because of the greater temperature tolerance of duckweed species. Both duckweed and water hyacinth systems have most often been used for nutrient removal after secondary treatment (IWA, 2000). Duckweed have several characteristics which place them in a high potential category for use in a wastewater treatment, for instance: they are among the fastest growing plants in the world, the plant has no woody tissue to maintain, and it responds to nutrient enrichment, converting various materials into high quality plant tissue. Duckweed, compared to water hyacinth, play a less role in the treatment process as they lack extensive root systems and therefore provide a smaller surface area for attached microbial growth. The main use of duckweeds is therefore in recovering nutrients from secondary treatment wastewater (Brix, 1993). A dense cover of duckweeds on the surface of water inhibits both, oxygen entering the water by diffusion, and the photosynthetic production of oxygen
by phytoplankton because of the poor light penetration. The water becomes largely anoxic, which in turns potentiate denitrification (Vymazal et al., 1998).

Suspended solids, BOD and pathogen removal mechanisms are the same encountered in conventional facultative stabilisation ponds. Nitrogen can be removed by direct plant uptake with frequent biomass harvesting, ammonia volatilization and microbial nitrification-denitrification. Nitrification of ammonia occurs within the aerobic root zone of duckweed. However, this layer is very thin and therefore little nitrification occurs in duckweed-based systems. Denitrification occurs in the much larger reduced zones in the water column. However, the nitrification-denitrification complex is not very effective for nitrogen removal because of limited nitrification in the system. Phosphorus may be removed either by direct plant uptake or by chemical precipitation in a separate treatment step (Vymazal et al., 1998).

The use of duckweed for wastewater treatment is not as well developed as hyacinth systems even though that first attempts to this plants were carried out 30 years ago (Vymazal et al., 1998).

1.3) FWS treatment wetlands with floating-leaved, bottom rooted macrophytes

Some macrophytes are rooted in the soils under the wetland waters, but their leaves float on the surface of the water (see Figure 3.5). Water lilies (Nymphaea spp.), lotus (Nelumbo spp.) and cowlily (Nuphar spp.) are all capable of this growth mode. Some treatment systems have been operated in this fashion; e.g., the lotus cell in the wetland treatment system in Bainikeng, China. Nymphaea and Nuphar are common in more open water systems, such as the Des Plaines River demonstrations near Wadsworth, Illinois, USA (IWA, 2000).

![Figure 3.5 Diagram of FWS wetland containing rooted, floating leaf plants (IWA, 2000).](image)

1.4) FWS treatment wetlands with floating mats

Some emergent wetlands macrophytes are capable of forming floating mats, even though their individual plants are not capable of such existence (see Figure 3.6). Cattails (Typha spp.), giant sweetgrass (Glyceria maxima), pennyworth (Hydrocotyle umbellata) and common reed (Phragmites australis) are capable of growing in mates (IWA, 2000).
Wastewater wetlands can accumulate large amounts of plant litter. This was so for surface flow wetlands planted with *Glyceria maxima*, which grows rapidly and has a high leaf turnover rate. Although this plant is normally established by planting it in soil in the bottom of wetland, within two years most of the plants were rooted in a floating mat of decaying leaf litter on the wetland surface. This mat grew to a thickness of more than 200 mm (IWA, 2000).

Cattails (*Typha* spp.) can also form floating mats in treatment wetlands. Rhizomes and roots become woven together and accumulate plant detritus to form the mat. The mat is stable as long as it retains sufficient areal extend. If small portions of the ‘raft’ detach, the plant are top-heavy and tip over (IWA, 2000).

### 1.5) FWS treatment with submersed macrophytes

Submerged aquatic plants such as waterweed (*Elodea* spp.), water milfoil (*Myriophyllum* spp.) and naiads (*Najas* spp.) have sometimes been used to treat wastewater. These submersed plants are buoyant and suspend in the water column, might or might not be rooted in the sediments (see Figure 3.7). Typically, most of the photosynthetic plant tissue is suspended in the water column, but many submersed plants have aerial portions that extend above the surface of water for flowering and increase light availability (IWA, 2000).
well in oxygenated water and therefore cannot be used in wastewater with a high content of readily-biodegradable organic matter because the microbial decomposition of the organic matter will create anoxic conditions (Brix, 1993). In addition, the turbidity of the water must not be too high to prevent light transmission to the plants to support their photosynthetic activity. The prime potential use of submerged macrophyte-based wastewater treatment is therefore for “polishing” secondarily treated wastewater, although good treatment of primary domestic effluent has been obtained in an Elodea nuttallii-based system (Vymazal et al., 1998).

The mechanisms by which submerged plants are able to remove ammonia from the water column is related to their high photosynthetic rates, which add oxygen to the water column, thus facilitating nitrification, and the fact that they utilise carbon dioxide from the water, thus raising the pH and driving ammonia to its volatile unionised form that can diffuse into the atmosphere. At night these plants respire (that is, use oxygen) in competition with the aquatic fauna. This category of constructed FWS wetlands has not had widespread usage, but purposely planted submersed plant species are present in many natural treatment wetlands and are invaders in constructed wetlands that have deep-water zones (IWA, 2000).

2) Subsurface flow treatment wetlands

Many of the earliest treatment wetlands in Europe were SSF systems constructed to treat mechanically pre-treated municipal wastewater. Soil- and gravel-based SSF wetlands are still the most prevalent application of this technology in Europe. This technology is generally limited to systems with low flow rates and can be used with less than secondary pre-treatment (IWA, 2000). Subsurface flow treatment wetlands may be classified according to the position of the drainage system, they can be horizontal- or vertical-flow systems.

2.1) Horizontal-flow systems

Figure 3. 8 shows a typical arrangement for a constructed wetland, with a horizontal flow (HF). It is called horizontal flow because the wastewater is fed at the inlet and flows slowly and through the porous medium, under the surface of the bed in a more or less horizontal path, until it reaches the outlet zone, where it is collected and discharged at the outlet. During this passage, the wastewater will come into contact with a network of aerobics, anoxic and anaerobic zones. The aerobic zones occur around roots and rhizomes, that leak oxygen into the substrate. During the passage of the wastewater through the rhizomes spheres, the wastewater is cleaned by microbiological degradation and by physical and chemical processes (IWA, 2000). The most common plant used in this constructed wetland is common reed (Phragmites australis). However, reed canary-grass (Phalaris arundinacea), sweet mannagrass (Glyceria maxima) and cattalis (Typha spp.) are also used in Europe.

Bacteria attached to plants underground organs (that is, roots and rhizomes) and media surfaces degrade both organic compounds, aerobically, as well as anaerobically. The oxygen required for aerobic degradation is supplied directly from the atmosphere by diffusion or oxygen from the macrophyte roots and rhizome in the rizosphere. Numerous
investigations have shown that the oxygen transport capacity of the reed is insufficient to ensure aerobic decomposition in the rhizosphere and that anoxic and anaerobic decomposition are important in HF constructed wetlands (IWA, 2000).

Settled solids that are not removed in pre-treatment systems are effectively removed by filtration and settlement. Settlement will take place in quiescent areas of any HF constructed wetland (Cooper et al., 1996).

Nitrogen is removed in HF constructed wetlands by nitrification and denitrification volatilization, adsorption and plant uptake. The major removal mechanism of nitrogen in HF constructed wetlands is nitrification and denitrification. Ammonia is oxidized to nitrate by nitrifying bacteria in aerobic zones, and nitrates are converted to gaseous nitrogen by denitrifying bacteria in anoxic zones (Cooper et al., 1996). Field measurements have shown that the oxygenation of the rhizosphere of HF constructed wetlands is insufficient and that incomplete nitrification is therefore the major cause of limited nitrogen removal. Volatilization, plant uptake and adsorption are much less important in nitrogen removal (IWA, 2000).

Phosphorus is removed from wastewater in HF wetlands primarily by ligand exchange reactions, in which phosphate displaces water or hydroxyl ions from the surface of Fe, Al or Ca, and therefore the removal of phosphorus is generally low (IWA, 2000).

2.2) Vertical-flow systems

Vertical flow wetlands comprise a flat bed of gravel topped with sand. They are normally planted with common reed and with the same densities as HF systems, however other emergent wetland plants such as cattails or bulrush can also be used. Figure 3. 9 shows a typical VF reed bed. They are fed intermittently. The liquid is dosed on the bed in a large batch, flooding the surface. The liquid then gradually drains vertically down through the bed and is collected by a drainage network at the base. The bed drains completely free, allowing air to refill the bed. The next dose of liquid traps this air and this together with the aeration caused by the rapid dosing on the bed leads to good oxygen
transfer (Cooper *et al*., 1996) and hence the ability to decompose organic matter and to nitrify ammonia nitrogen.

![Figure 3.9](image)

**Figure 3.9** System with vertical subsurface water flow (percolation), the species illustrated is *Phragmites australis* (Brix, 1993).

As with the HF systems, the reeds in VF systems will transfer some oxygen down into the rhizosphere, but it will be small in comparison with oxygen transfer created by the dosing system (Cooper *et al*., 1996).

VF treatment wetlands are very similar in principle to a rustic biological filter (Cooper *et al*., 1996). They are not as good for the removal of TSS and in most cases will be followed by a HF bed as part of a multistage treatment wetland system (IWA, 2000).

The earliest form of VF system is that of Seidel in Germany in the 1970s, sometimes called the Max Planck Institute Process (MPIP) or the Krefeld Process. Interest in the particular process seemed to wane, but it has been revived in the past six years because of the need to produce beds that nitrify. Operators and designers were disappointed in the ability of the early HF systems to oxidise ammonia to nitrate. In retrospect, this was clearly related to the fact that the ability of the reeds to transfer oxygen was greatly overestimated. Most HF systems have very low levels of dissolved oxygen in the effluent. Under these circumstances there will be no oxygen remaining to oxidise the ammonia nitrogen to nitrate. Because of this poor performance, designers and researchers started looking for alternative designs of reed beds that could oxidise the ammonia nitrogen (IWA, 2000).

### 3.2.2. Removal processes in constructed wetlands.

Constructed wetlands are able to reduce many contaminants, including organics (BOD and COD), suspended solids, nitrogen, phosphorus, trace metals, and pathogens. This reduction is accomplished by diverse treatment mechanisms as it is shown in the Table 3.1.
A description of the performances capabilities of both types of constructed wetland will be started written latter. Although vertical flow constructed wetlands will be described in detail since the experimental plant belongs to this group.

1) Suspended solids

Wetlands systems have long hydraulic residence times, generally several days or longer. Consequently, most settleable or flotable solids of wastewater origins are removed. The major processes responsible for removal of settleable suspended solids are sedimentation and filtration (Vymazal et al., 1998). Non-settling/colloidal solids are removed, at least partially, by bacterial growth due to the weight increase of these, and collision with adsorption to other solids (plants, pond bottom, suspended solids, etc.).

In all types of constructed wetlands most of the solids of wastewater origin are filtered out and settled within the first few meters beyond the inlet zone. The accumulation of trapped suspended solids is a major threat for good performance of some systems, especially those with subsurface flow, which can be clogged with suspended solids, especially in VF constructed wetlands. However, and based on experiments carried out by Bavor and Schulz in Australia (1993), between 80 and 90% of both primary and secondary influent solids were volatile. In systems with primary treatment, however, the majority of settleable solids are removed in a mechanical pre-treatment unit (e.g. sedimentation or
Imhoff tanks) before the wastewater is discharged to the wetland system (Vymazal et al., 1998).

2) Organic compounds

Settleable organics are rapidly removed in wetland system under quiescent conditions by deposition and filtration. Attached and suspended microbial growth is responsible for removal of soluble organics. Organic compounds are degraded aerobically as well as anaerobically. The oxygen required for aerobic degradation is supplied directly from the atmosphere by diffusion or leakage from the macrophyte roots into the rhizosphere. Uptake of organic matter by the macrophytes is negligible compared to biological degradation (Vymazal et al., 1998).

Aerobic degradation is governed by aerobic heterotrophic bacteria according to the following reaction:

\[(\text{CH}_2\text{O})_n + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O}\]  

(3.1)

Under aerobic conditions, also it is possible that an autotrophic group of bacteria will degrade organic compounds containing nitrogen. This group of bacteria is called nitrifying bacteria and the process is called ammonification. Cooper et al. (1996) pointed out that both groups consume organics but the faster metabolic rate of the heterotrophs means that they are mainly responsible for the reduction in the BOD of the system.

Anaerobic degradation is a multi-step process which occurs within the constructed wetlands in the absence of dissolved oxygen (Cooper et al., 1996). The process can be carried out by either facultative and obligate anaerobic heterotrophic bacteria. In the first step the primary end-products of fermentation are fatty acids such as acetic (Eq. 2), butyric, and lactic acids (Eq. 3), alcohols (Eq. 4) and the gases CO2 and H2 (Vymazal 1995).

\[\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{CH}_3\text{COOH} + \text{H}_2\]  

(3.2)

\[\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{CH}_3\text{CHOHCOOH} \quad \text{(lactic acid)}\]  

(3.3)

\[\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{CO}_2 + 2 \text{CH}_3\text{CH}_2\text{OH} \quad \text{(ethanol)}\]  

(3.4)

Acid acetic is the primary acid formed in most flooded soils and sediments. Strictly anaerobic sulphate-reducing (Eq. 5) and methane-forming (Eqs. 6 and 7) bacteria then utilize the end-products of fermentation and, in fact, depends on the complex community of fermentative bacteria to supply substrate for their metabolic activities. Both groups play an important role in organic matter decomposition and carbon cycling in wetlands (Vymazal et al., 1998).

\[\text{CH}_3\text{COOH} + \text{H}_2\text{SO}_4 \rightarrow 2 \text{CO}_2 + 2 \text{H}_2\text{O} + \text{H}_2\text{S}\]  

(3.5)

\[\text{CH}_3\text{COOH} + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}\]  

(3.6)
The acid-forming bacteria are fairly adaptable but the methane-formers are most sensitive and will only operate in the pH range 6.5 to 7.5. Overproduction of acid by the acid-formers can rapidly result in low pH, thus stopping the action of the methane-forming bacteria and resulting in production of odorous compounds from the constructed wetland. Anaerobic degradation of organic compounds is much slower than aerobic degradation. However, when oxygen is limiting at high organic loadings, anaerobic degradation will predominate (Cooper et al., 1996).

3) Nitrogen

The removal mechanisms for nitrogen are numerous and include volatilization, ammonification, nitrification-denitrification, plant uptake and adsorption. Studies have proven that the major removal mechanism in most constructed wetlands is microbial nitrification-denitrification (Vymazal et al., 1998).

Both processes nitrification and denitrification are defined in the section 3.3 as well as the nitrogen cycle and the effect of recycling treated wastewater in these processes.

4) Phosphorus

Phosphorus is typically present in wastewater as orthophosphate, polyphosphate and organic phosphorus. Biological oxidation results in the conversion of most phosphorus in orthophosphate forms. Phosphorus removal in wetland treatment systems occurs from adsorption, plant absorption, complexation and precipitation (Vymazal et al., 1998).

Most wetlands studies have shown that wetlands are no particularly effective as phosphorus sink when compared with terrestrial ecosystems. The phosphorus cycle and the processes involved in it are developed in the section 3.4.

5) Metals

The processes of metal removal in constructed wetlands include sedimentation, filtration, adsorption, complexation, precipitation, cation exchange, plant uptake, and microbially-mediated reactions, especially oxidation (Vymazal et al., 1998).

Metals occurs in either the soluble or particulate associated forms, with the former representing the most bio-available form, particularly when the metal is present as either an ionic or weakly complexed species. Physico-chemical processes such as sorption, precipitation, complexation, sedimentation, erosion and diffusion determine the distribution between particulate and dissolved phases. Specific parameters which control the sediment-water partitioning of metals include flow/suspended solids ratio, oxic/anoxic conditions, ionic strength, pH value, dissolved and particulate organic carbon contents, organic and inorganic ligand concentrations and metal mobilisation by biochemically-mediated reactions (Cooper et al., 1996).
6) Pathogens

Bacteria and viruses are important organisms from a public health point of view. Constructed wetlands known to offer a suitable combination of physical, chemical and biological factors for the removal of pathogenic organisms. Physical factors include mechanical filtration and sedimentation. Chemical factors include oxidation, UV radiation, exposure to biocides excreted by some plants and adsorption to organic matter. Biological removal mechanisms include antibiosis, predation by nematodes, protests and zooplankton, attack by lytic bacteria and viruses and natural die-off (Vymazal et al., 1998).

3.2.3. Thermal effects

Wetland water temperatures can influence some pollutant removal and conversion processes. Although best prospects for successful wetland treatment are in the warm regions, studies in North America and Scandinavia (Kadlec and Knight, 1996) show that treatment may be feasible also in cool regions. In this climates ice formation can influence system hydraulics and other engineering, but many treatment processes proceed in under-ice flow.

Cold climate is taken as equivalent to “cold temperate” climate, where the coldest month has a mean temperature below -3°C and the warmest a mean above 10°C. This classification includes most of Canada, Alaska and northern contiguous USA, Scandinavia, Eastern Europe, Russia and north-eastern China. In the particular case of Denmark, which is just to the south of cold temperate region, this is a world-pioneer country in promoting SSF wetlands. The Danish database contains information about 109 systems, which most of them treat municipal wastewater (about Denmark’s case will be described in the section 3.2.4).

In summer, energy gains are primarily from solar radiation and to a smaller extent from warm air (they can be losses for cool air) and a small portion of this recharges the soil energy storage. Losses are primarily to evapotranspiration and to a minor extent to the cool deep soils. In winter, gains are from soil storage, and loss is to the cold ambient air. At the snow surface, radiation, convection and sublimation create a balance that dictates the snow surface temperature. During periods of ice cover, treatment wetlands are typically just above freezing, 0-2°C.

Temperature swings at the wetland outlet can be as large as 10°C from day to day because of the strong influence of meteorological factors. There is also a diurnal cycles in water temperature, with daytime temperatures ranging from 5 to 15 °C higher that nighttime temperatures.

Snow and ice

During the frozen season, the presence of insulating layers of snow and ice change the application of the energy balance considerably. Energy gains are now solely from deep soil storage, and losses are by heat conduction through the snow and ice to the cold air above and to air formation. Incoming sensible heat is typically dissipated because
are generally greater than gains. Evaporation from the water layer is prevented by the ice cap. If heat losses are severe enough, ice formation occurs (IWA, 2000). Experience has shown that ice will begin to form on a water surface when the water temperatures reaches 3°C, because of density differences and convective losses. Water is shallow and unstratified, so the effluent temperature for freezing conditions is close to 0°C. Heat generation due to oxidation of BOD and plant detritus is a very small but positive contribution to the energy balance.

The amount of ice formation is determined by climate conditions that vary greatly from one winter to another. The presence of some ice on treatment wetlands can be a benefit in that the ice layer provides insulation and slows the cooling of the underlying water. Ice thicknesses in wetlands are therefore much less than in adjacent lakes or frost depths in nearby uplands (IWA, 2000). It might be beneficial to operate the wetland with a higher water level at the time of freezing and thus create a space for both water and air beneath the ice (Cabello, 2002).

Ice that forms in the upper horizons on an SSF bed is physically supported by the medium. Consequently, water levels can be dropped to create an air zone in the matrix above the water and below the ice. These various forms of insulation combine to allow winter operation of SSF wetlands in extremely cold climates (IWA, 2000).

Hydrological and hydraulic conditions

Cold climate with snow will influence the wetland water balance as a result of dry winter periods with snow accumulation and little run-off, followed by snow melt and flooding during spring. During snowmelt, the hydraulic residence time (HRT) may decrease dramatically. In such a situation it is advisable to provide for storage, so that the peak loads from the catchments and the sewers can be separated (Cabello, 2002).

Production of biofilm can increase due to environmental stress during temperatures changes. Increased thickness of the biofilm due to a fall in temperature and decreased oxygen availability in winter-time may lead to decrease hydraulic conductivity in SSF treatment systems. However, results from the literature on whether the biofilm will increase or decrease during low temperature, are contradictory (Wittgren and Mæhlum, 1997).

Biological and biochemical processes

Nutrient uptake by plants and microbial transformations of wastewater components and plants litter in wetlands are both directly and indirectly affected by climatic conditions. Directly in the sense that plant physiology is governed by solar radiation and temperature, and microbial processes by temperature alone. The indirect influences include the dependence of biological and biochemical processes on physical conditions, which in turn are affected by climatic conditions. The HRT, oxygen availability and freezing/thawing of soil are important examples of such physical conditions (Wittgren and Mæhlum, 1997).

In Denmark, a temperature coefficient around two is typical for many biological processes, which means that the bacterial activity doubles when the temperature is
increased by 10 degrees. For this reason, it may be expected that the degradation of organic matter and nitrification-denitrification (being microbial processes) will be significantly slower during the winter period (Brix, 1998)

3.2.4 A particular case: wetlands in Denmark

Since the early eighties, there has been an ample quest to find appropriate and economically reasonable solutions for sewage treatment for small wastewater producers in Denmark. The traditional solution would be to collect the sewage from several small producers and conduct it to a central conventional wastewater treatment facility. However, a centralised solution is rather expensive, and the municipalities have therefore been investigating the possibility of finding alternative less-expensive on-site solutions. A number of different solutions has been launched as soil infiltrations systems, sandfilters, pond systems and reed beds (Brix, 1998).

Full-scale constructed reed beds have been in operation in Denmark since 1983. All the public reed beds have discharge standards or outlet criteria defined by the counties. Normally the owners of constructed wetlands have the obligation to undertake 4 to 12 quality control analyses throughout the year (Brix, 1998).

Several municipalities accepted the theoretical assumptions for the operation of this kind of macrophyte-based wastewater treatment system concerning the reduction of different parameter values. For all the parameters an efficiency better than 90% was claimed when a reed bed surface area of 3 to 5 m² PE⁻¹ (PE = person equivalent) was assumed. In all Danish reed beds the wastewater is pre-treated before inlet to the reed beds and, generally, this pre-treatment takes place in two- or three-chamber settling tanks. The shape of these systems has been evolving and the latest generation system consists of two stages: horizontal-flow beds based on sand followed by pulse-loaded vertical flow units (Brix, 1998).

All constructed reed beds have been excavated to a depth of 0.6-1 m. In most cases, a water tight membrane of polyethylene, PVC, or clay has been placed at the bottom of the excavation in order to prevent seepage and contamination of the ground-water. In most cases local soil has been used as the medium in the reed beds, but in a few cases the soils have been amended with sand, organic material or chalk in order to improve the hydraulic permeability or the ability to retain phosphorus. The pre-treated sewage is generally led to a stone-filled inlet trench by one or more water pipes in order to secure a good distribution of the sewage in the trench. In many cases the inlet is distributed through a drainage pipe buried in the inlet trench. At the outlet a similar stone-filled is used to collect water from the reed beds. In other way the constructed wetlands are usually planted with *Phragmites australis* (Common Reed) (Brix, 1998).

After the introduction of the technology in Denmark in 1983 the interest in the technique was high shortly. The high interest prevailed until 1988 where a number of new constructions decreased (see Figure 3.10). The main reason was a new legislation (The Water Environment Plan) which defined new requirements to nutrient removal for large producers of wastewater (>5000 PE). In the following years until 1992, the municipalities
therefore had to concentrate their investments on the major sources of wastewater, and less emphasis was put on small on-site systems. Furthermore, the fact that the earlier systems did not fulfil the initial expectations to nutrient removal resulted in generally decreasing interest in constructed reed bed systems (Brix, 1998).

The vast majority of Danish reed beds are constructed to treat domestic sewage from small villages in rural areas and as on-site systems for single households and farms. Constructed reed beds are also used to treat wastewater from schools and institutions, camping sites, leachate from solid waste deposits, run-off from roads, and effluent from some industries (Brix, 1998).

The criteria for reed beds – besides demands for temperature, pH and oxygen saturation – in general include discharge standards for TSS (< 20 or 30 mg l\(^{-1}\)) and BOD\(_5\) (< 10, 15 or 20 mg l\(^{-1}\)). A number of systems discharging into sensitive small streams and rivers have discharge standards for ammonium. At sites where the final recipient is a lake, some reed beds have discharge standard for phosphorus (Brix, 1998). But in general quality control analyses include measurements of pH, total suspended solids (TSS), biochemical oxygen demand (BOD), dissolved oxygen, chemical oxygen demand (COD), total nitrogen (total-N), nitrite and nitrate (NO\(_2^-\) + NO\(_3^-\)), ammonium nitrogen (NH\(_4^+\)-N), total phosphorus (total-P) and orthophosphorus (PO\(_4^{3-}\)-P) (Brix, 1998).

The data base of inlet and outlet quality data from 90 Danish systems are summarised in the following lines:

**Total Suspended Solids (TSS)**

The influent concentrations of TSS varied between < 2 to 3800 mg l\(^{-1}\) (median 75 mg l\(^{-1}\)) depending on the type of wastewater and the efficiency of the mechanical pre-treatment. 92% of all systems had median effluent concentrations standard of 20 mg l\(^{-1}\) and 76% had effluent concentrations below 10 mg l\(^{-1}\). Effluent concentrations exceeding this value were often connected with production of algae in systems with open water in the effluent channel. The effluent concentrations were independent of the inlet concentrations as well as the TSS mass loading rate and the hydraulic loading rate (Brix, 1998).
Biochemical Oxygen Demand (BOD)

The median inlet concentration of BOD₅ was 94 mg l⁻¹ varying from less than 10 to > 8000 mg l⁻¹. About a third of all systems had median effluent concentrations of < 5 mg l⁻¹, 80% had < 10 mg l⁻¹, and 98% had median effluent D0B₅ concentrations of < 20 mg l⁻¹. The effluent concentration of BOD₅ was largely independent of inlet concentration as well as on the mass loading and the hydraulic loading rate. The BOD₅ mass loading rate of the systems varied between < 1 to 23 g BOD₅ m⁻² day⁻¹ (Brix, 1998).

pH

There was no tendency to a pH reduction during the passage through the reed beds, which in case could have influenced the stability of precipitated phosphorus compound within the systems. The median pH in both inlet and outlet was ~7.6, and the inlet pH varied more than the effluent pH. A decrease in pH could have resulted from nitrification. However, the buffer capacity of the water is normally high and a decrease in pH due to nitrification may not be detected. In all systems the average effluent pH during the whole period of operation was > 7 (Brix, 1998).

Nitrogen

A linear relationship between inlet and effluent concentration of total-N was observed. The regression coefficient (r² = 0.58) indicates a mean removal efficiency of ~50% for total-N. There were no clear relationships between the effluent concentration of total-N and the mass loading rate and the removal efficiency was independent of inlet concentration. The median inlet and outlet concentrations of total-N were 35 mg l⁻¹ (range 2.1 - >300 mg l⁻¹) and 20 mg l⁻¹ (range 0.7 – 198 mg l⁻¹), respectively (Brix, 1998).

Nitrogen occurs primarily as NH₄-N in the inlet and outlet water, the median inlet and outlet concentration being 25 and 12 mg l⁻¹, respectively. In general the removal of NH₄-N was low in the reed beds, and the effluent concentrations of NH₄ were dependent on the inlet concentrations. As expected, most reed beds had low inlet concentrations of NO₂ + NO₃ (median 1 mg l⁻¹). A number of systems had higher inlet concentrations of NO₃, and in those systems there was a tendency to lower effluent concentrations as a consequence of denitrification. Under optimal conditions, ammonium should be nitrified during the passage of the reed beds, and the produced NO₂ and NO₃ subsequently denitrified. Nitrification seems to be the process limiting nitrogen removal (Brix, 1998).

Phosphorus

The inlet concentration of total-P varied between 0.04 and 175 mg l⁻¹ (median 7.4 mg l⁻¹) and the effluent concentration between 0.01 and 55 mg l⁻¹ (median 4.8 mg l⁻¹). The results show that the removal of total-P varies between systems, probably as a consequence of differences in system designs, in spite of the regression coefficient (r² = 0.42) indicates a mean removal efficiency of total-P of ~54%. However in most of the systems the removal of total-P was less, maybe 20-30%, and some systems had much better performance. There were no clear relationship the effluent concentration of total-P and the mass loading rate, and the percent removal was independent of the inlet concentrations (Brix, 1998).
If an analyzes of how performances varies of the previous parameters as a function of age it is possible to see that the removal efficiency increased for TSS. In most of the systems, the stabilisation of the TSS effluent concentration, were connected with the development of a dense vegetation. This means that after only one growing season the systems worked satisfactory. In the same way, the average effluent concentrations of BOD$_5$ decreased during the first years and the removal efficiency increased up to 95%. The average effluent concentration of total-P improved during the working years, but this improvement was higher in the latest years. The removal efficiency reached a mere 50%. The pattern for total-N resembled that of total-P (removal efficiency increased up to 55%).

In Danish reed beds the temperature of the water and the medium vary at least 10°C between summer and winter. The data shows that the mean water temperature varies between less than 5°C in December through February to more than 15°C in July-August. Therefore, it may be expected that the degradation of organic matter and nitrification-denitrification – being microbial processes – will be significantly slower during the winter period. The strength of the wastewater also varies through the season, with higher concentrations during summer than during winter and this can be explained by the fact that many systems receive rainwater in addition to the sewage water. The variation in wastewater strength therefore reflects the seasonal distribution of rain in Denmark. The effluent concentrations of especially P and N vary in concert with the inlet concentrations, thus, the removal efficiencies, expressed as a percentage removal, remains throughout the year. It can be therefore concluded that despite the fact that the temperature of the water varies by more than 10 degrees between summer and winter in Denmark, there is no seasonal variation in treatment performances. The microorganisms in the reed beds may slowly adapts, both qualitatively and quantitatively, to the seasonal changes in temperature and thereby maintain the bacterial activity throughout the winter. Especially the rate of microbial nitrification and denitrification processes are temperature sensitive. If coupled nitrification and denitrification were major removal mechanisms for nitrogen in the reed beds, a significant seasonal effect in treatment performance would be expected. However, no seasonal changes in nitrogen removal were observed, indicating that nitrification and denitrification are not the most important removal processes for nitrogen. The dominant process for removal of nitrogen and phosphorus seems therefore to be depositions in the reed bed, a process that is independent of the temperature (Brix, 1998).

### 3.3 Nitrogen

In the latest the nutrients removal of wastewater, such as nitrogen and phosphorus, has been necessary to control more frequently due to theirs potentials impacts on the quality of the water. The presence of nitrogen in a wastewater discharge can be undesirable for several reasons:

- Nitrogen compounds in both organic form or ammonium use an amount of dissolved oxygen that can reduce it below the necessary levels to fishes and others animals.
- Nitrites and ammonium are extremely toxic to fish and many other aquatic organisms.
Nitrogen in all of its forms can be available as a nutrient to aquatic plants and consequently contribute to eutrophication. Nitrate ion it is a potential public health hazard in water consumed by infants.

Municipal wastewater of predominantly domestic origin contains nitrogen in the organic and ammonium forms. These are primarily waste products originating from protein metabolism in the human body. In fresh sewage, about 60 percent of the nitrogen is in organic form and 40 percent in the ammonium form. Bacterial decomposition of proteinaceous matter and hydrolysis of urea transform organic nitrogen to the ammonium form. Normally, very little (less than 1 percent) of the nitrogen in the fresh sewage is in the oxidized form of nitrate or nitrite (Sedlak, 1991).

The average daily per capita production rate of nitrogen is approximately 16 grams. The nitrogen concentration in a wastewater depends on the per capita wastewater flow rate (Sedlak, 1991). Table 3.2 shows the typical values of nitrogen in a wastewater.

**Table 3.2** Typical nitrogen composition of fresh sewage (Metcalf & Eddy, 1995).

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>Weak</th>
<th>Medium</th>
<th>Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen</td>
<td>20</td>
<td>40</td>
<td>85</td>
</tr>
<tr>
<td>N-organic</td>
<td>8</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Ammonium</td>
<td>12</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Nitrites</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Danish EPA gives typical values of flow rates, BOD$_5$ and total nitrogen in order to help the sizing of small constructed wetlands (for a treatment of up to 30 PE). This ones are shown in the Table 3.3.

**Table 3.3** Typical inlet values for constructed wetlands (Vejledning fra Miljøstyrelsen, 1999).

<table>
<thead>
<tr>
<th>Number of persons equivalent</th>
<th>Flow rate (m$^3$/day)</th>
<th>BOD$_5$ (g/day)</th>
<th>Total-N (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>0,75</td>
<td>300</td>
<td>65</td>
</tr>
<tr>
<td>6-10</td>
<td>1,5</td>
<td>600</td>
<td>130</td>
</tr>
<tr>
<td>11-15</td>
<td>2,25</td>
<td>900</td>
<td>195</td>
</tr>
<tr>
<td>16-20</td>
<td>3,0</td>
<td>1200</td>
<td>260</td>
</tr>
<tr>
<td>21-25</td>
<td>3,75</td>
<td>1500</td>
<td>325</td>
</tr>
<tr>
<td>26-30</td>
<td>4,5</td>
<td>1800</td>
<td>390</td>
</tr>
</tbody>
</table>

Industrial and commercial contributions, ground garbage and storm water will affect the nitrogen concentrations in raw wastewater, being the storm water an important parameter in Denmark since dilutes the N-concentration, as well as other parameters.
3.3.1 The nitrogen cycle and its processes

Nitrogen exists in many forms because of the high number of oxidation states it can assume. In ammonium and organic nitrogen compounds, which are the forms most closely associated with plants and animals, its oxidation state is -3. At the other extreme, when nitrogen is in the nitrate form, its oxidation is +5 (see Table 3.4). In the environment, changes to one oxidation state to another can be accomplished biologically by living organisms. The most prevalent forms of nitrogen in wastewaters and, therefore, those which many require treatment, are organic, ammonium and nitrate nitrogen (Sedlak, 1991).

### Table 3.4 Different forms of nitrogen in the wastewater.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oxidized state</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_2$-N</td>
<td>-3</td>
<td>Organic Nitrogen</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>-3</td>
<td>Ammonia</td>
</tr>
<tr>
<td>N$_2$</td>
<td>0</td>
<td>Nitrogen Gas</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>+3</td>
<td>Nitrite</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>+5</td>
<td>Nitrate</td>
</tr>
</tbody>
</table>

Nitrogen transformations that may occur in biological treatment systems are illustrated in the Figure 3.11. In this one is possible to see that organic nitrogen present in raw wastewaters may be transformed to ammonia through bacterial decomposition of proteinaceous matter and hydrolysis urea. In any biological treatment system some bacterial growth always takes place. Since nitrogen constitutes 12 to 13 percent of cell dry mass, some ammonia nitrogen will be assimilated in newly formed cells. Hence, part of the ammonia used for cell synthesis will be returned to the liquid through lysis and autoxidation (Sedlak, 1991).

![Diagram of nitrogen cycle](image)

**Figure 3.11** Simplified wetland nitrogen cycle (Kadlec & Knight, 1996).
Under appropriate conditions ammonia nitrogen can be oxidized in two-step process to form nitrates. Two groups of microorganisms (nitrifiers) in the presence of oxygen carry out this process, called nitrification. Finally, nitrates may be transformed to nitrogen gas through a process called denitrification. This transformation is accomplished by denitrifying microorganisms in the absence of oxygen. An organic carbon source is required for denitrification to occur. The nitrogen gas formed escapes to the atmosphere (Sedlak, 1991).

The variations of nitrogen forms in time are shown in the Figure 3. 12. This is one way to see which compounds are the most important to take care to optimize the nitrogen removal process.

![Figure 3. 12 Biological nitrogen conversion processes (Sedlak, 1991).](image)

Below, the processes concerning the nitrogen cycle are developed and focused on the constructed wetland systems although the current bibliography available about nitrification and denitrification processes is mainly based on activated sludge or in horizontal sub-surface flow.

**Ammonification**

Ammonification is the biological transformation of organic nitrogen to ammonia and is the first step in mineralization of organic nitrogen. This process occurs through microbial breakdown of organic tissues containing amino acids, through excretion of ammonia directly by plants and animals, and by hydrolysis of urea and uric acid (Kadlec & Knight, 1996). Mineralization rates are fattest in the oxygenated zone, and decrease as mineralization switches from aerobic to facultative anaerobic and obligate anaerobic microflora. The rate of ammonification in wetlands is dependent on temperature, pH value, C/N ratio of the residue, available nutrients in the system, and soil conditions such as texture and structure. The optimum pH range for the ammonification process is between 6.5 and 8.5 (Vymazal et al. 1998). Reddy et al. (1979) concluded from the literature data that the rate of aerobic ammonification doubles with a temperature increase of 10°C.
Ammonia volatilisation

Ammonia volatilisation is a physicochemical process where ammonium N is known to be in equilibrium between gaseous and hydroxyl forms as indicated below:

\[
\text{NH}_4^+ \, ? \, \text{NH}_3 + \text{H}^+ \quad (3.8)
\]

\[
k = \frac{[\text{NH}_3][\text{H}^+]}{[\text{NH}_4^+]} \quad (3.9)
\]

\[
\text{pK} = -\log_{10} k = 9.4 \quad (3.10)
\]

Some studies pointed out that losses of NH\textsubscript{3} through volatilisation from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often losses are not serious if the pH is below 8.0. At pH of 9.3 the ratio between ammonia and ammonium ion is 1:1 and the losses via volatilisation are significant. Algal photosynthesis in constructed wetlands as well as photosynthesis by free-floating submerged macrophytes often creates high pH values (Vymazal et al., 1998).

Based on a broad literature review Vymazal (1995) summarized that volatilisation rate is controlled by the NH\textsubscript{4}+ concentration in the water, temperature, wind velocity, solar radiation, the nature and number of aquatic plants, and the capacity of the system to change the pH value in diurnal cycles (absence of CO\textsubscript{2} increases volatilisation).

Nitrogen removal by assimilation

Since nitrogen is an essential constituent of microbial cells, any net growth of biomass that is removed from the waste stream will cause some nitrogen removal. The amount of nitrogen that can be removed by this mechanism is limited by the amount of net growth, which in turn depends on the carbonaceous organic content of the wastewater and the system’s operating conditions. Since nitrogen content of microbial cells is approximately 12.5 percent (on a dry weight basis), the amount of nitrogen that will be removed by assimilation will be:

\[
\frac{\text{dNH}_3 - \text{N}}{\text{dt}} = 0.125 \cdot \frac{\text{dX}_v}{\text{dt}} \quad (3.11)
\]

where : \(\text{dNH}_3\text{-N}/\text{dt}\) : rate of nitrogen removed by assimilation, mg/day.
\(\text{dX}_v/\text{dt}\) : rate of active biomass or biological sludge production, mg/day.

In an activated sludge system, the nitrogen removal by assimilation is limited to approximately 2 to 5 percent of the raw wastewater BOD, depending on operating conditions. Based on primary effluent BOD and nitrogen concentrations of 120 and 30 mg/l, respectively, the percent nitrogen removal in the treatment of domestic wastewater may range from 8 to 20 percent. This removal mechanism may become quite significant in wastewater having relatively high concentrations of BOD, such as in some industrial wastewater or municipal wastewaters with a large industrial contributor (Sedlak, 1991).
constructed wetlands these values only will be true when the system starts because of the microorganisms are growing so invest all the matter to develop themselves and increase theirs volume. After the growing period these bacteria reduce the activity because only needs BOD and nitrogen to maintain the activity, so the nitrogen percent removal will range under activated sludge percent.

It is important to be aware that when a large portion of assimilated nitrogen returns to a waste stream from sludge handling processes, particularly heat treatment and anaerobic digestion, the overall nitrogen removal will be less (Sedlak, 1991). In constructed wetlands once the system is stabilized it could be possible to hypothesise that the net removal by assimilation from nitrifier and denitrifier bacteria is seldom zero.

Nitrification

Nitrification is the biological oxidation of ammonia to nitrite and subsequently from nitrite to nitrate. The microorganisms involved in fresh waters are *Nitrosomonas* and *Nitrobacter*. Nitrification is a chemoautotrophic process. The nitrifying bacteria derive energy from the oxidation of ammonia and/or nitrite and carbon dioxide is used as a carbon source for synthesis new cells. These organisms require O$_2$ during ammonium-N oxidation to nitrite-N and nitrite-N oxidation to nitrate-N. The nitrification is carried out in the following two steps:

$$2\ NH_4^+ + 3\ O_2 \rightarrow Nitrosomonas \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+ + \text{Cells} \quad (3.12)$$

$$2\ NO_2^- + O_2 \rightarrow Nitrobacter \rightarrow 2\ NO_3^- + \text{Cells} \quad (3.13)$$

And, therefore, the global reaction can be expressed by the following equation:

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O + \text{Cells} \quad (3.14)$$

The first step, the oxidation of ammonia to nitrite, is executed by strictly chemolithotrophic (strictly aerobic) bacteria, *Nitrosomonas*, which are entirely dependent on the oxidation of ammonia for the generation of energy for growth. The probable sequence for the oxidation of ammonia to nitrite by Nitroso group bacteria is:

ammonia (NH$_3$/NH$_4^+$) \rightarrow hydroxylamine (NH$_2$OH) \rightarrow nitroxy1 (NOH) \rightarrow nitrohydroxylamine (NH$_2$.NH$_2$OH) \rightarrow nitrite (NO$_2^-$) \quad (3.15)

The second step in the process of nitrification, the oxidation of nitrite to nitrate, is performed by facultative chemolithotrophic bacteria, *Nitrobacter*, which can also use organic compounds, in addition to nitrite, for the generation of energy for growth.

Vymazal (1985) summarized that nitrification is influenced by temperature, pH value, alkalinity of water, inorganic C source, microbial population and concentration of ammonium-N and dissolved oxygen.
There are two important aspects to see in the Eq. (14): first of all the high levels of oxygen that the reaction needs and, secondly, how the medium is becoming more and more acid due to 1 mol of N- NH\textsubscript{4}\textsuperscript{+} gives 2 moles of H\textsuperscript{+}.

Since a buildup of nitrite is rarely observed (see Figure 3.12), it can be concluded that the rate of conversion to nitrate controls the rate of overall reaction (Sedlak, 1991).

The extent of nitrification that occurs during treatment is dependent on the extend to which nitrifying organisms are present. The cell mass comprised of nitrifying organism is referred here as the nitrifier’s volatile suspended solids (NVSS). The cell yield for *nitrosomonas* has been reported as 0.05 - 0.29 g NVSS/g NH\textsubscript{3}-N and *nitrobacter* 0.02 – 0.08 g NVSS/g NO\textsubscript{2}-N. A value of 0.15 g NVSS/g NH\textsubscript{3}-N oxidized is usually used for design purposes (Sedlak, 1991). The empirical overall reaction including oxidation and synthesis is:

\[
\text{NH}_4^+ + 1.83 \text{O}_2 + 1.98 \text{HCO}_3^- ? 0.98 \text{NO}_3^- + 0.021 \text{C}_5\text{H}_7\text{NO}_2 + 1.88 \text{H}_2\text{CO}_3 + 1.04 \text{H}_2\text{O} \tag{3.16}
\]

Thus, the stoichiometric for nitrification indicates that for one gram of ammonia nitrogen removed approximately:

- 4.33 g of O\textsubscript{2} are consumed.
- 7.14 g of alkalinity (as CaCO\textsubscript{3}) are destroyed.
- 0.15 g of new cells are formed
- 0.08 g of inorganic carbon are consumed

In wastewater with low alkalinity and/or high ammonia concentrations, alkalinity may have to be added in order to maintain the pH at the optimum level of nitrification. The effect of the pH on the nitrification reaction is shown in Figure 3.13. As shown the figure, over the range of pH 7.0 to 8.0 there is little effect on nitrification rate. Since the pH of municipal wastewater usually falls within this range, pH should not be a factor.

![Figure 3.13 Effect of pH on ammonia oxidation (Sedlak, 1991).](image)
However, it is especially important that there be sufficient alkalinity in the wastewater to balance the acid produced by nitrification or else alterations in pH could have an adverse effect on nitrification. As indicated above, about 7.14 mg of alkalinity (as CaCO$_3$) are consumed per mg NH$_3$-N oxidized. This means that municipal wastewater with 50 mg TKN/l available for oxidation should have an alkalinity of about 400 mg/l (as CaCO$_3$) to ensure a residual of 40 mg/l (as CaCO$_3$) after full nitrification. The consumption of alkalinity has a depressing effect on the pH. Nitrification reduces the HCO$_3^-$ concentration and increases the H$_2$CO$_3$ concentration. This effect is mediated by CO$_2$ stripping duration aeration. If the CO$_2$ is not stripped from the liquid, as can occur in high purity oxygen systems, the alkalinity may have to be as much as 10 times greater that the amount of ammonia nitrified. As shown later, this problem is alleviated when denitrification is employed since one-half of the alkalinity is recovered in the denitrification process (Sedlak, 1991).

Once the nitrification reactions are known it is possible to begin studying the characteristics of the bacteria responsible for the process. The Monod expression provides a convenient relationship for predicting bacterial growth rate based on the concentration of a limiting substrate, the maximum potential rate when that substrate is not limiting, and the half saturation constant K for that substrate:

\[ \mu_A = \mu_{A,\text{max}} \frac{S}{S + K} \]  

(3.17)

where:
- $\mu_A$: specific growth rate
- $\mu_{A,\text{max}}$: maximum specific growth rate
- $S$: substrate concentration in the bioreactor
- $K$: is the half saturation coefficient for the substrate

Besides oxygen and micronutrients, the limiting substrate for nitrifier growth is ammonia for *Nitrosomonas* and nitrite for *Nitrobacter*. Nitrite generally converts to nitrate faster than ammonia converts to nitrite, so the sequential oxidation of ammonia to nitrate is controlled kinetically by the slow first step, the *Nitrosomonas* bacterial growth rate (Kadlec and Knight, 1996). Furthermore, some experimental studies has given the following values, depending on the temperature, in activated sludge systems (Hernández, 1997):

*Nitrosomonas*: $\mu_{\text{max}} = 0.47 \cdot 1.10^{(T-15)}$

*Nitrobacter*: $\mu_{\text{max}} = 0.78 \cdot 1.06^{(T-15)}$

where T is the temperature of the wastewater.

These values are valid for a temperature range between 8°C and 30°C, although this would imply that under Denmark climatic conditions the value sometimes cannot be valid, but it shows that the specific growth of *Nitrosomonas* is the critical factor. The minimum temperature for growth of *Nitrosomonas* is 5°C and *Nitrobacter* is 4°C (Cooper *et al*, 1996), being the maximum growth rate of nitrifiers at 15°C where the maximum specific growth rate can reaches 0.45 day$^{-1}$ (Sedkal, 1991).
Antoniou et al. (1990) affirm that the maximum specific growth rate of nitrifying bacteria is the most crucial parameter describing the growth of the autotrophic biomass. The nitrification capacity of a wastewater treatment plant cannot be determined without a correct assessment of this parameter. Since the maximum specific growth rate is a strong function of the prevailing temperature and pH, a functional relationship accounting for this dependence could be used to evaluate a plant’s performance.

On the other hand, a considerable effort has been expended to research the factors that influence nitrification in conventional wastewater treatment systems. Most work has examined nitrification in suspended growth, activated sludge systems. However, nitrification kinetics also have been documented in conventional attached growth systems such as trickling filters and rotating biological contactors. Less experimental work has been published concerning nitrification kinetics in wetlands. (Kadlec and Knight, 1996).

The actual growth rate of nitrifiers in an activated sludge, as well as in constructed wetlands, system is dependent on the concentration of ammonia nitrogen and dissolved oxygen (DO). Dissolved oxygen concentrations of above 1 mg/l are also required below which oxygen becomes the limiting substrate for growth (Cooper et al., 1996). The effects of DO and effluent ammonia are expressed by a Monod kinetic expression (Sedlak, 1991):

$$\mu_N = \left(\frac{\text{NH}_3-N}{K_N + \text{NH}_3-N}\right)\left(\frac{\text{DO}}{K_0 + \text{DO}}\right)$$

(3.18)

where: $\mu_{N,\text{max}}$ : maximum specific growth rate of nitrifiers, g NVSS/g NVSS-day

$K_N$ and $K_0$ : half saturation coefficients for nitrogen and oxygen, respectively, mg/l

Typical value of $K_N$ is 0.3 mg/l in activated sludge and the $K_0$ has been reported to vary from 0.2 to 1.0 in these systems (Sedlak, 1991). In activated sludge and depending on the loading rate, and taking into account that the introduction of the oxygen is through discrete points, sometimes parts of the flocs do not receive the necessary amount of oxygen, so the process is not the optimum. In vertical flow constructed wetlands, as explained in the section 3.2.1, before the primary effluent is dosed into the bed it is full in fully aerated conditions and therefore theoretically completely aerobic. The bed is fed by a large batch, flooding the surface, and the liquid gradually drains vertically down through the gravel and the bacteria takes the substrate (nitrogen) in aerobic conditions.

pH-value, as it has been commented before, is able to vary the specific growth rate of nitrifiers. Nitrification tends to lower pH in the water column by forming carbonic acid; however, this pH reduction can be buffered by the loss of carbon dioxide from the water column to the atmosphere. The WPCF (1983) nutrient control manual recommends that pH be maintained above 7.2 for nitrification stability, and the U.S. EPA (1993) provides a formula for estimating the nitrification rate at pH values between 6.0 and 7.2:

$$\mu_N = \mu_{N,\text{max}} (1 - 0.833 (7.2 - \text{pH}))$$

(3.19)

Monod-type limiting equations can be multiplied to determine the reaction rate based on multiple variables. Combining Eq. (18) and Eq. (19) provides an expression for
nitrification rate in suspended growth treatment systems based on ammonia nitrogen concentration, dissolved oxygen and pH:

\[
\mu_N = (\mu_{N,\text{max}}) \left[ 1 - 0.833(7.2 - pH)\left( \frac{\text{NH}_4 - N}{K_N + \text{NH}_3 - N} \right) \left( \frac{\text{DO}}{K_0 + \text{DO}} \right) \right]
\]  

(3.20)

The specific nitrifier growth rate is directly proportional to the specific rate of nitrification based on a nitrifier yield coefficient (Kadlec and Knight, 1996):

\[
U_N = \mu_N / Y_N
\]  

(3.21)

where:  
\(U_N\): specific rate of nitrification, g NH\(_3\)-N/g NVSS-day  
\(Y_N\): nitrifier yield coefficient, g NVSS/g NH\(_3\)-N

And from an empirically derived effective nitrifier mass per wetland surface area, \(V_N\), the former expression for nitrification can be presented as the following (Kadlec and Knight, 1996):

\[
J_N = U_N V_N = (\mu_N V_N) / Y_N
\]  

(3.22)

where:  
\(V_N\): effective nitrifier mass per wetland surface are, g/m\(^2\)  
\(J_N\): the nitrification rate in g NH\(_4\) – N/m\(^2\)/yr

Furthermore, it is necessary to consider that the nitrifier bacteria are not the only ones present in the system, there other autotrophic and heterotrophic bacteria. In all domestic and in most industrial wastewaters, the concentration of carbonaceous organics greatly exceeds that of nitrogen. The heterotrophic organisms yield also exceeds that of the autotrophs. Hence, the autotrophic population normally constitutes a small fraction of the total biomass. Neglecting the endogenous decay process, the nitrifier’s (autotrophs) fraction can be estimated by the equation (Sedlak, 1991):

\[
F_N = \frac{(a_N) \cdot (A_r)}{(a) \cdot (S_r) + (a_N) \cdot (A_r)}
\]  

(3.23)

where:  
\(F_N\): nitrifier fraction  
\(a\): heterotrophs yield coefficient, g VSS/g BOD  
\(A_r\): ammonia nitrogen removed, mg/l  
\(S_r\): BOD removed, mg/l

Therefore, the nitrification rate can be expressed in terms of the total biomass concentration and the nitrifier fraction:

\[
R_N = (U_N)(F_N)(X_N)
\]  

(3.24)

where:  
\(R_N\): nitrification rate in g NH\(_4\) – N/m\(^2\)/yr.  
\(X_N\): concentration of nitrifiers, mg/l
The nitrification capacity of a system equals the product of the nitrogen removal rate ($R_N$) and the detention time. Transient peak loading conditions allowable are the sum of a system’s nitrification capacity and the allowable discharge concentration.

But even though the former expression, Kadlec and Knight (1996) affirm that wetland microcosm data do not support the Monod model, because ammonium disappearance is apparently first order rather than zero order at higher concentrations. Moreover, the application of the Eq. (20) and (22) to treatment wetlands is impractical for several reasons. First, the prediction of the specific yield per unit area of wetland ($V_N/Y_N$) is not presently possible. Second, wetlands promote attached growth processes to the near exclusion of suspended growth processes. Finally, treatment wetlands house other processes by which ammonium losses and gains may occur: nitrification in oxygenated microzones in the rhizosphere, plant uptake and biomass decay, and the above-water decomposition of plant material. Mineralization of organic nitrogen produces ammonium. Thus, even if wetland biofilm processes are describable as an attached growth nitrification system, other processes can prevent accurate prediction of ammonium loss. The rates of ammonium reduction in treatment wetlands are presently predictable only from wetland data.

An alternative expression that can be used to model the disappearance of ammonia (nitrification and other processes combined) in wetlands treatment systems is the area-based, first-order model. Hence, the overall process of ammonia loss with a zero background can be expressed as the following:

$$R_N = k_{AN} C_{AN}$$

(3.25)

where :

- $R_N$ : ammonia disappearance rate in g NH$_4$ – N/m$^2$/yr
- $k_{AN}$ : area-based, first-order ammonia disappearance rate constant, m/yr
- $C_{AN}$ : concentration of ammonia, mg/l

The integrated form of ammonia mass balance for this uptake rate and plug flow is:

$$\ln \left[ \frac{C_{AN,0}}{C_{AN}} \right] = \frac{-k_{AN}}{q} \cdot y = -k_{VAN} \cdot T \cdot y$$

(3.26)

where :

- $k_{VAN}$ : first-order volumetric NH$_4$ – N reduction rate constant, l/d
- $q$ : hydraulic loading rate, m/yr
- $t$ : detention time, days
- $y$ : fractional distance through the wetlands.

On the other hand, Gijs and Lesley (1994) explained that nitrification process may be achieved by heterotrophic bacteria as well. Heterotrophic nitrifiers oxidize a range of reduced nitrogen compounds, apparently without gaining energy from the reaction. There are certainly two distinct pathways involved, the inorganic pathway of nitrification, Eq. (25), and the organic, Eq. (26):

$$\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$$

(3.27)
These researchers assure that there are a large number of heterotrophs present in both natural and man-made systems which might make a significant contribution under certain conditions. Indeed, Castignetti and Gunner (1980) had shown that ammonium-oxidizing heterotrophs were capable of producing sufficient \( \text{NO}_2^- \) to support growth of a nitrobacter species. Measurements based on nitrogen balances revealed that, while still lower per unit of biomass than autotrophic nitrification rates, heterotrophic nitrification rates were at least an order of magnitude higher than previously believed. Hence, if the biomass made up of heterotrophic nitrifiers was 20-50 timer higher than that of autotrophic nitrifiers, the total conversion rates of the heterotrophic nitrifiers could easily match those of the autotrophs. In order to examine the influence of two potentially critical parameters: \( \text{O}_2 \) and C:N ratio, and to obtain quantitative data on the relative importance of autotrophic nitrification and the heterotrophic combination of nitrification and denitrification, competition experiments between a representative heterotrophic nitrifier (\( T. \ pantotropha \)) and a representative autotrophic ammonium oxidizer (\( N. \ europaea \)) were therefore set up (see Figure 3. 14).

![Figure 3. 14 Fate of \( \text{NH}_4^+ \) in co-cultures of \( T. \ pantotropha \) and \( N. \ europaea \) at different C:N ratios (Gijs and Lesley, 1994).](image)

The results show that the amount of nitrogen is directly proportional to the amount of \( N. \ europaea \) and indirectly proportional to the amount of \( T. \ pantotropha \). On the other hand, the higher the C:N ratio is, the higher is the heterotrophic nitrification and nitrogen removal by assimilation. When the ratio is around double fold the autotrophic nitrification predominantes whereas when the C:N ratio is 10 times the heterotrophic nitrification is the predominant.

**Factors affecting nitrification**

Nitrifying organisms are subject to inhibition by various organic compounds. Hockenbury and Grady (1977) have summarized inhibition data for selected organic compounds as shown in Table 3. 5 and Table 3. 6.
If inhibitor compounds are present in wastewater, then the performances of separate stage or combined systems will probably be different. In a separate stage system, the inhibitor substance will probably be degraded in the first stage and second stage kinetics will proceeded normally. The performances of a combined system may be poorer because of reduced degradation on the inhibitors substance. The nitrification will proceed normally when inhibitors are degraded. However if the concentration of the inhibitory substance is sufficiently high it is possible to inhibit nitrification completely. In this case, nitrification will not proceed until the inhibitory substance is degraded. In many industrial wastewater or municipal wastewaters with a high industrial input, the rate of nitrification is sharply reduced.

Table 3.5 Effects of organic compounds on degree of inhibition of ammonia oxidation (Sedlak, 1991).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Degree of inhibition at the concentration (mg/L) indicated, %</th>
<th>Estimated concentration giving 50% inhibition (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowsoylamine</td>
<td>66, 66, 66, 66, 66b, 66c, 66d, 66e</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Aniline</td>
<td>56, 56, 56, 56, 56, 56, 56, 56c</td>
<td>&lt;1</td>
</tr>
<tr>
<td>n-Methylaniline</td>
<td>50, 50, 50, 50, 50, 50, 50, 50c</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1-Naphthylaniline</td>
<td>61, 61, 61, 61, 61, 61, 61, 61c</td>
<td>15</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>73, 73, 73, 73, 73, 73, 73, 73c</td>
<td>17</td>
</tr>
<tr>
<td>2,2'-Bipyridine</td>
<td>91, 91, 91, 91, 91, 91, 91, 91c</td>
<td>23</td>
</tr>
<tr>
<td>p-Nitroaniline</td>
<td>94, 94, 94, 94, 94, 94, 94, 94c</td>
<td>31</td>
</tr>
<tr>
<td>p-Aminophenolphenone</td>
<td>80, 80, 80, 80, 80, 80, 80, 80c</td>
<td>43</td>
</tr>
<tr>
<td>Benzidine di-HCl</td>
<td>84, 84, 84, 84, 84, 84, 84, 84c</td>
<td>45</td>
</tr>
<tr>
<td>p-Phenylazaniline</td>
<td>54, 54, 54, 54, 54, 54, 54, 54c</td>
<td>72</td>
</tr>
<tr>
<td>Hexamethylene diamine</td>
<td>52, 52, 52, 52, 52, 52, 52, 52c</td>
<td>85</td>
</tr>
<tr>
<td>p-Nitrobenzaldehyde</td>
<td>76, 76, 76, 76, 76, 76, 76, 76c</td>
<td>87</td>
</tr>
<tr>
<td>Naphthylhydrazine</td>
<td>30, 30, 30, 30, 30, 30, 30, 30c</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>30, 30, 30, 30, 30, 30, 30, 30c</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Dimethylglyoxime</td>
<td>30, 30, 30, 30, 30, 30, 30, 30c</td>
<td>&gt;140</td>
</tr>
<tr>
<td>Benzylamine</td>
<td>26, 26, 26, 26, 26, 26, 26, 26c</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>20, 20, 20, 20, 20, 20, 20, 20c</td>
<td>&gt;150</td>
</tr>
<tr>
<td>Monosulfonamide</td>
<td>16, 16, 16, 16, 16, 16, 16, 16c</td>
<td>&gt;200</td>
</tr>
<tr>
<td>*Industrially significant chemicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 1 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 2.5 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 5 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 10 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 150 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a = 200 mg/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Denitrification**

The first anoxic oxidation process to occur after oxygen depletion is the reduction of nitrate to molecular nitrogen or nitrogen gases (N₂, N₂O and NO). From a biochemical viewpoint, denitrification is a bacterial process in which nitrogen oxides (in ionic or gaseous forms) serve as terminal electron acceptors for respiratory electron transport. Electrons are carried from an electron-donating substrate (usually, but not exclusively, organic compounds) through several carrier systems to a more oxidized N form. The
resultant free energy is conserved in ATP, following phosphorylation, and used by the denitrifying organisms to support respiration (Vymazal et al., 1998).

**Table 3.6** Organic compounds that inhibit activated sludge nitrification (Sedlak, 1991).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration* (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone†</td>
<td>2000.000</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>19.500</td>
</tr>
<tr>
<td>Allyl chloride</td>
<td>180.000</td>
</tr>
<tr>
<td>Allyl isothiocyanate</td>
<td>1.900</td>
</tr>
<tr>
<td>Benzo[b]thiazole disulfide</td>
<td>39.000</td>
</tr>
<tr>
<td>Carbon disulfide†</td>
<td>35.000</td>
</tr>
<tr>
<td>Chloroform†</td>
<td>18.000</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>12.800</td>
</tr>
<tr>
<td>Diallyl ether</td>
<td>100.000</td>
</tr>
<tr>
<td>Dichlorodiamide</td>
<td>250.000</td>
</tr>
<tr>
<td>Diguandine</td>
<td>50.000</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>460.000</td>
</tr>
<tr>
<td>Dithiooximide</td>
<td>1.100</td>
</tr>
<tr>
<td>Ethanol†</td>
<td>2400.000</td>
</tr>
<tr>
<td>Guanidine carbonate</td>
<td>18.500</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>58.000</td>
</tr>
<tr>
<td>6-Hydroxyquinoline</td>
<td>72.500</td>
</tr>
<tr>
<td>Mercaptobenzothiazole</td>
<td>3.000</td>
</tr>
<tr>
<td>Methylamine hydrochloride</td>
<td>1550.000</td>
</tr>
<tr>
<td>Methyl isothiocyanate</td>
<td>0.800</td>
</tr>
<tr>
<td>Methyl thionuronium sulfate</td>
<td>6.500</td>
</tr>
<tr>
<td>Phenol†</td>
<td>5.800</td>
</tr>
<tr>
<td>Potassium thiocyanate</td>
<td>306.000</td>
</tr>
<tr>
<td>Skatol</td>
<td>7.000</td>
</tr>
<tr>
<td>Sodium dimethyl dithiocarbamate</td>
<td>13.600</td>
</tr>
<tr>
<td>Sodium methyl dithiocarbamate</td>
<td>0.900</td>
</tr>
<tr>
<td>Tetramethyl thiuram disulfide</td>
<td>30.000</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>0.530</td>
</tr>
<tr>
<td>Thiocarbamazide</td>
<td>0.180</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>0.076</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>118.000</td>
</tr>
</tbody>
</table>

*Concentration giving approximately 75% inhibition.  
†Industrially significant chemicals.

Denitrifying ability has been demonstrated in 17 genera of bacteria. Most denitrifying bacteria are chemoheterotrophs. They obtain the energy solely through chemical reactions and use organic compounds as electron donors and as a source of cellular carbon. The genera Bacillus, Micrococcus and Pseudomonas are probably the most important in soils; Pseudomonas, Aeromonas and Vibrio in the aquatic environments. When oxygen is available, these organisms oxidize a carbohydrate substrate to CO$_2$ and H$_2$O. Aerobic respiration using oxygen as an electron acceptor or anaerobic respiration using nitrogen for this purpose is accomplished by the denitrifier with the same series of electron transport system. This facility to function both as an aerobe and as an anaerobe is of great practical importance because it enables denitrification to proceed at a significant rate soon after the onset of anoxic conditions (a redox potential of about 300 mV) without change in microbial population. Because denitrification is carried out almost exclusively
by facultative anaerobic heterotrophs that substitute oxidized N forms for O₂ as electron acceptors in respiratory processes, and because these processes follow aerobic biochemical routes, it can be misleading to refer to denitrification as an anaerobic process. It is rather one process that takes place under anoxic conditions (i.e. when there is no DO present but where oxygen is available from sources such as nitrate, nitrite or even sulphate) (Vymazal et al., 1998).

Denitrification is illustrated by following equation (Vymazal et al., 1998):

\[
6 \text{(CH}_2\text{O)} + 4 \text{NO}_3^- \rightarrow 6 \text{CO}_2 + 2 \text{N}_2 + 6 \text{H}_2\text{O}
\]  

(3.29)

This reaction is irreversible, and occurs in the presence of available organic substrate only under anaerobic or anoxic conditions, where nitrogen is used as an electron acceptor in place of oxygen. More and more evidence is being provided from pure culture studies that nitrate reduction can occur in the presence of oxygen (Vymazal et al., 1998). Moreover, Kadlec and Knight (1996) affirm that denitrification has been observed in suspended and attached growth treatment systems that have relatively low measured DO concentration. This observation is explained by the presence of microscopic, anoxic zones that are likely to occur in bacterial films.

Gaseous N production during denitrification can also be depicted by:

\[
4 \text{(CH}_2\text{O)} + 4 \text{NO}_3^- \rightarrow 4 \text{HCO}_3^- + 2 \text{N}_2 + 2 \text{H}_2\text{O}
\]  

(3.30)

\[
5 \text{(CH}_2\text{O)} + 4 \text{NO}_3^- \rightarrow \text{H}_2\text{CO}_3 + 4 \text{HCO}_3^- + 2 \text{N}_2 + 2 \text{H}_2\text{O}
\]  

(3.31)

The stoichiometric reaction describing denitrification depends on the carbonaceous matter involved. For methanol, which is the most extensively used and studied external carbon source, the reaction is (Sedlak, 1991):

\[
6 \text{NO}_3^- + 5 \text{CH}_3\text{OH} \rightarrow 3 \text{N}_2 + 5 \text{CO}_2 + 7 \text{H}_2\text{O} + 6 \text{OH}^-
\]  

(3.32)

Including cell synthesis the empirical reaction is (Sedlak, 1991):

\[
\text{NO}_3^- + 1.08 \text{CH}_3\text{OH} + 0.24 \text{H}_2\text{CO}_3 \rightarrow 0.06 \text{C}_5\text{H}_7\text{NO}_2 + 0.47 \text{N}_2 + 1.68 \text{H}_2\text{O} + \text{HCO}_3^-
\]  

(3.33)

This reaction expression indicates that for one gram of nitrate-N that is denitrified:

- 2.47 g of methanol (or approximately 3.7 g of COD) are consumed.
- 0.45 g of new cells are produced
- 3.57 g of alkalinity are formed

In the former expression is possible to see how 3.57 g of alkalinity is formed, so this can result in rises in pH value, being the optimum range that one that is between 7 and 8 (Cooper et al. 1996). Experimental studies in conventional systems indicate that denitrification is considerably reduced at a pH less than 6 and greater than 8. Treatment
wetlands at circumneutral pH provide ideal environments in terms of hydrogen ion concentrations (Kadlec and Knight, 1996).

Nitrate will also replace oxygen in the endogenous respiration reaction. The proposed equation is:

\[ C_5H_7NO_2 + 4.6 \text{NO}_3^- \rightarrow 5 \text{CO}_2 + 2.8 \text{N}_2 + 4.6 \text{OH}^- + 1.2 \text{H}_2\text{O} \quad (3.34) \]

The rate of denitrification depends primarily on the nature and concentration of the carbonaceous matter undergoing degradation. Most investigators agree that denitrification is a zero order reaction with respect to nitrate down to very low nitrate concentration levels. Hence the nitrate removal is an anoxic basin when carbon is not limiting can be expressed by:

\[ (\text{NO}_3^-)_o - (\text{NO}_3^-)_e = (R_{DN})(X_V)(t) \quad (3.35) \]

where:  
- \((\text{NO}_3^-)_o\) and \((\text{NO}_3^-)_e\): influent and effluent nitrate-N, respectively, mg/l  
- \(R_{DN}\): zero order rate denitrification, g NO\(_3\)-N/g VSS-day  
- \(X_V\): mixed liquor volatile suspended solids concentration, mg/l  
- \(t\): hydraulic detention time, day

Values of \(R_{DN}\) for various carbon sources are given in the Table 3.7 Denitrification rates with various carbon sources (Sedlak, 1991).

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Denitrification Rate (g NO(_3)-N/g VSS-day)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.21 to 0.32</td>
<td>25</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.12 to 0.90</td>
<td>20</td>
</tr>
<tr>
<td>Sewage</td>
<td>0.03 to 0.11</td>
<td>15-27</td>
</tr>
<tr>
<td>Sewage</td>
<td>0.072 to 0.72</td>
<td>--</td>
</tr>
<tr>
<td>Endogenous Metabolism</td>
<td>0.017 to 0.048</td>
<td>12-20</td>
</tr>
</tbody>
</table>

The presence of dissolved oxygen suppresses the enzyme system needed for denitrification and is a critical parameter. This process is also strongly temperature dependent and only proceeds at very low rates, if at all, at temperatures below 5°C (Cooper et al., 1996). Sedlak (1991) proposes the following equation to consider both effects:

\[ R_{DN(T)} = R_{DN(T)} K^{(T-20)}(1-\text{DO}) \quad (3.36) \]

Values of \(K\) range from 1.03 to 1.1. A value of 1.09 is commonly used. The denitrification rate will depend on both the concentration and the biodegradability of the carbon source (Sedlak, 1991).

Analogue to oxygen utilization in aerobic systems, the denitrification rate can be expressed by:

\[ (\text{NO}_3\text{-N})_t = (A'N)(S_t) + (b'N)(X_{vt}t) \quad (3.37) \]
where: $A_N$: nitrate utilization in anoxic degradation, g NO$_3$-N/g BOD
$S_r$: BOD removed, mg/l.
$X_{DN}$: concentration of denitrifiers, g VSS-day
$b'_N$: nitrate utilization in endogenous respiration under anoxic conditions, g NO$_3$-N/g VSS-day

Treating municipal wastewater, Sedlak (1991) explains how Barnard (1975) found three distinct denitrification rates, as shown in Figure 3.15. The first rate of 50 mg/l-h lasted from 5 to 15 minutes and was attributed to by-products from anaerobic fermentation. The second rate of 16 mg/l-h, was attributed to normal assimilation of the particulate and more complex compounds, and lasted until all external food sources were exhausted. The third rate of 5.4 mg/l-h was attributed to endogenous respiration.

The denitrification rate under aerobic conditions will depend on the anoxic fraction of the biological floc and the availability of carbon substrate. The DO term in the equation (36) indicates that the denitrification rate decreases linearly to zero when the dissolved oxygen concentration reaches 1.0 mg/l. Further research is necessary to establish the actual denitrification rate versus dissolved oxygen levels. Denitrification rates of 0.006 mg NO$_3$-N/mg VSS/day have been reported under aerobic conditions. Sedlak (1991) advises that for practical purposes denitrification can be ignored when dissolved oxygen concentrations are greater than 1 mg/l.

![Figure 3.15 Sequential denitrification rates (Sedlak, 1991).](image)

**Plant uptake**

The potential rate of nutrient uptake by plant is limited by its net productivity (growth rate) and the concentration of nutrients in the plant tissue. Nutrient storage is similarly dependent on plant tissue nutrient concentrations, and also on the ultimate potential for biomass accumulation: that is, the maximum standing crop. Therefore,
desirable traits of a plant used for nutrient assimilation and storage would include rapid growth, high tissue nutrient content, and the capability to attain a high standing crop (biomass per unit area) (Vymazal et al. 1998)

Few data has been reported for plants established in constructed wetlands treating wastewaters. In addition, it is important to note that the amount of nutrients that can be removed by harvesting in secondary treatment systems are generally insignificant compared to the loading into the constructed wetlands with the wastewater (Brix 1994). This is especially true for constructed wetlands with emergent macrophytes. It has been reported that under optimum conditions the amount of nitrogen removed with the biomass can achieve 10-16% of the total removed nitrogen. The removal of nutrients through harvesting may play more important role in treatment systems designed for polishing purposes (Vymazal et al. 1998).

In the literature, there are many reviews on nitrogen concentrations in plant tissue as well as nitrogen standing stocks for plants found in natural stands. The uptake capacity of emergent macrophytes, and thus the amount that can be removed if the biomass is harvested, is roughly in the range 1000-2000 kg N ha\(^{-1}\) yr\(^{-1}\). The highly productive Water Hyacinth have higher uptake capacity (up to nearly 6000 kg N ha\(^{-1}\) yr\(^{-1}\)) whereas the capacity of submerged macrophytes is lower (about 700 kg N ha\(^{-1}\) yr\(^{-1}\)) (Vymazal et al. 1998).

If the wetland is not harvested, the vast majority of the nutrients that have been incorporated into the plant tissue will be returned to the water by decomposition process. Long-term storage of nutrients in the wetland systems results from the undecomposed fraction of the litter produced by various elements of the biochemical cycles as well as deposition of refractory nutrient-containing compounds (Brix, 1996).

3.3.2 The effect of the recycling. Previous experiences.

It has been accepted that the recycling of the effluent can increase the efficiency for nitrogen removal in a constructed wetland system. The system has to have two different zones, where aerobic and anoxic conditions will be able to develop themselves. As it has been explained in the previous section, the first step has to be nitrification and when the whole nitrogen is in oxidized nitrogen state, it is possible that the denitrification will occur. Vertical flow constructed wetlands efficiency in producing well nitrified is widely documented (Cooper, 1998; IWA, 2000; Brix et al., 2002). Horizontal flow constructed wetlands and other systems are capable to develop anoxic conditions, which are necessary for the denitrification process to occur.

Not much literature is found in the topic that concerns us, although Kantawanichkul et al (2001), and Kantawanichkul et al. (2002) have publish a couple articles which I presented below.
Optimum ratio ratio for nitrogen removal in a combined system: vertical flow vegetated bed over horizontal flow sand bed (Kantawanichkul et al, 2001).

In Thailand, livestock farming industry has recently undergone a rapid growth and constructed wetlands have been introduced to threat pig farm effluent, following anaerobic digestion, but due to insufficient knowledge and experience, these systems have not been yet effective due to high concentration of ammonia in the wastewater. However, constructed wetlands have the potential to remove nitrogen if a system composed of a vertical flow (which nitrify) and of a horizontal flow (which denitrify) will be performed.

The system showed in the Figure 3.16 was constructed, in order to assess the efficiency of constructed wetlands for nitrogen removal. The plant presented consists of a tank separated in two section by a plastic sheet: in the upper section a vertical flow bed was placed. This VF was fed intermittently (4 hours on and 4 hours off), and the effluent was collected at the bottom with a pipe and conducted to the lower section, where the water loaded into HF horizontally. Four separate runs where carried out with the following recycling percentages from the lower section to the upper: 0%, 50%, 100% and 200%.

![Figure 3.16 The experimental design.](image)

The run without recycling was conducted with a hydraulic loading rate of 3.7 cm d\(^{-1}\). The average of COD was 2688.7 mg l\(^{-1}\) and after passing through the vertical bed the removal efficiency reached 97.8%, and after the horizontal bed the efficiency further increased to 98.3% (the average effluent was 56.5 mg l\(^{-1}\)). The TKN concentration in the inlet was 242.4 mg l\(^{-1}\). After the first bed the concentration was 6.7 mg l\(^{-1}\) and after the horizontal bed the concentration was 3.5 mg l\(^{-1}\) (removal of 97.1% and 98.5% respectively). The same pattern was observed for NH\(_3\)-N with a concentration of 156.3 mg l\(^{-1}\) in the raw wastewater, which was reduced in the final effluent to 1.7 mg l\(^{-1}\). The nitrate increased through the vertical bed from 0.7 mg l\(^{-1}\) to 73.4 mg l\(^{-1}\) followed by a reduction to 64.9 mg l\(^{-1}\) in the horizontal flow due to denitrification. The total removal of the system in term of total nitrogen reached 70.8%.
In the run with 50% of recycling the hydraulic loading rate was increased to 5.6 cm d\(^{-1}\). The average COD in the raw wastewater was 3301.5 mg l\(^{-1}\) and reduced to 1791.3 mg l\(^{-1}\) after mixing. The efficiency at the end of the process was around 97% (74 mg l\(^{-1}\) in the effluent). TKN and NH\(_3\)-N in the raw wastewater was 419.8 and 305.8 mg l\(^{-1}\) respectively, and after mixing, were diluted to 237.3 and 168.6 mg l\(^{-1}\). Both TKN and NH\(_3\)-N were reduced by 99%. The average nitrate in the influent wastewater was 1.1 mg l\(^{-1}\) and after mixing the concentration increased until 27.6 mg l\(^{-1}\). In the vertical bed, also increased to 142.3 mg l\(^{-1}\) that was reduced at the end of the system to 52.7 mg l\(^{-1}\) (or approximately 62.9% of removal).

With 100% of recycling, the hydraulic loading rate was then increased to 7.5 cm d\(^{-1}\). The concentration of TKN and NH\(_3\)-N in the raw wastewater was 313.8 and 237.9 mg l\(^{-1}\) in average, and after mixed was reduced to 170 and 126 mg l\(^{-1}\). In the first bed, the reduction was significant to 7.8 and 7.2 mg l\(^{-1}\) in average (97.5 and 97% removal efficiency). Oxidized nitrogen was 0.8 mg l\(^{-1}\) in the inlet of the system and increase after dilution and through the first bed to 81.7 mg l\(^{-1}\). This one was reduced in the horizontal flow bed to 16.1 mg l\(^{-1}\) in average (80.3% reduction). The removal of total nitrogen was 93% from the whole system. The concentration of COD was 2008.8 mg l\(^{-1}\) and diluted after mixing to 1083.4 mg l\(^{-1}\) in average. The 97.5% of COD was removed in the vertical flow bed and after passing the horizontal bed the removal efficiency was increased to 98.3%.

When the recycling ratio was increased to 200%, the hydraulic loading rate was 11.25 cm d\(^{-1}\). The concentration of TKN and NH\(_3\)-N in the raw wastewater was 305.1 mg l\(^{-1}\) and 243.2 mg l\(^{-1}\) in average. Oxidised nitrogen was 0.7 mg l\(^{-1}\). The concentration diluted mixed influent were 113.5, 91.5 and 13.5 mg l\(^{-1}\) in average of TKN, NH\(_3\)-N and NO\(_3\)-N, respectively. The concentrations of TKN and NH\(_3\)-N in the effluent were 26.4 and 23.4 mg l\(^{-1}\) in average (91.4% and 90.3% removal-efficiency). The concentration of oxidised nitrogen increased to 42.2 mg l\(^{-1}\) only and then reduced in horizontal flow bed to 22.6 mg l\(^{-1}\) (46.4% reduction). Therefore, the removal of total nitrogen was 85.2% from the whole system. The concentration of COD in the influent was 2149.4 mg l\(^{-1}\) and 769 mg l\(^{-1}\) in the diluted mix influent. The removal efficiency in the vertical flow bed was 94.4% and after passing the horizontal one, the efficiency increased to 95.1%.

Suspended solids were efficiently removed in every run (over 97%) due to filtration, sedimentation and biodegradation. Evaporation was observed in the vertical flow section. The experimental unit was located outdoor and exposed to sunlight. The water lost was approximately 63%.

The growth and nitrogen accumulation in the plants were measured and analysed for each run. The total nitrogen accumulation was 0.24, 0.66, 0.38 and 0.33 g m\(^{-2}\) d\(^{-1}\) respectively at the end of each run, respectively. However, nitrogen uptake by plants was very low (between 2.6 and 4% of TN input), compare to ammonia volatilisation and bacteria utilisation, which could not be measured in this study, but the estimation from TN input and output, it was about 60 – 84%.

All the removal efficiencies are summarised in the following Table:
Table 3.8. Removal efficiency of wastewater characteristics in the different runs (Kantawanichkul et al., 2001).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Run 1 (0%)</th>
<th>Run 2 (50%)</th>
<th>Run 3 (100%)</th>
<th>Run 4 (200%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS (%)</td>
<td>97.7</td>
<td>98.8</td>
<td>98.7</td>
<td>98.5</td>
</tr>
<tr>
<td>COD (%)</td>
<td>98.3</td>
<td>97.6</td>
<td>98.3</td>
<td>95.1</td>
</tr>
<tr>
<td>TKN (%)</td>
<td>98.5</td>
<td>98.9</td>
<td>98.2</td>
<td>92.7</td>
</tr>
<tr>
<td>NH₃-N (%)</td>
<td>98.8</td>
<td>99.2</td>
<td>98.0</td>
<td>91.9</td>
</tr>
<tr>
<td>NOₓ-N (%)</td>
<td>11.6</td>
<td>62.9</td>
<td>80.3</td>
<td>46.4</td>
</tr>
<tr>
<td>TN (%)</td>
<td>70.8</td>
<td>85.7</td>
<td>93.0</td>
<td>85.2</td>
</tr>
</tbody>
</table>

Kantawanichkul et al. (2001) concluded that the efficiency of the system, especially for nitrogen removal, was satisfactory. Recycling and nitrified effluent increased nitrogen removal efficiency and TN removal could reach 93% with 100% recycling ratio. Nitrification with intermittent feeding in vertical flow vegetated bed was very successful. Recycling of 100% of the effluent increased the denitrification from 13 to 80% compare with non-recycling. Over 95% removal efficiency of COD and 97% of suspended solids were achieved in every run and the recycling did not significantly improve further the removal efficiency. Biomass production of Cyperus and nitrogen were notably different with and without effluent recycling.

In other paper, Kantawanichkul et al. (2002) explains the effect of the hydraulic loading rate in the process of nitrification and denitrification, and the differences between two systems in the process of nitrification and denitrification which is summarized in the following lines.

Nitrogen removal by two experimental combined wetland system in a tropical climate (Kantawanichkul et al., 2002).

The experiment was composed of two-combined constructed wetland. System A was composed of a vegetated vertical sand bed over an un-vegetated horizontal flow sand bed (see Figure 3.17). Raw wastewater from an anaerobic treatment system was fed intermittently (4 hours on and 4 hours off) to the top of the lower section of the tank. The water infiltrated horizontally and across to the bottom of the tank and collected at the opposite site. The effluent pipe was raised in order to saturate the media, then fed to the surface of the upper section and flowed vertically down through to reach the bottom. The treated wastewater was collected by perforated pipes and recycled to the lower section of the system at 1:1 ratio, as Kantawanichkul et al. (2001) reported to be the optimum recycling ratio to enhance the nitrogen removal.
The system B was a vegetated subsurface horizontal flow followed by a vegetated vertical flow bed (see Figure 3.18). Wastewater was fed to the first tank via perforated PVC pipe and infiltrated horizontally down across to the bottom of opposite site. The water level was maintained to saturate the media in the horizontal flow bed and was then collected via a perforated PVC pipe and conveyed to the second tank by the perforated PVC pipes laid on the surface of the media. The water trickled down through the media to the bottom and was collected in a perforated pipe laid on the bottom. The effluent was recycled to the first tank with the ratio of 1:1.

These systems were tested under three different loading rates (in which were included raw wastewater and recycled effluent): 3, 6 and 12 cm/d.

For 120 days (June to September) the systems were tested under 3 cm/d hydraulic loading rate. The average temperature of the wastewater was 29°C and the pH 7.9. The
The concentration of TKN in the raw wastewater was 400 mg/l and after mixing with recycled effluent, the concentration was diluted to 227 mg/l. Nitrification was satisfactory in vertical flow vegetated bed and denitrification was also clearly noticeable in horizontal flow bed. Most of the TKN and NH$_3$-N concentration was reduced by nitrification in the vertical flow vegetated bed resulting in 76-85% removal efficiency in the final effluent of system A and 79-89% in the system B. In this experiment, oxidised nitrogen (NO$_x$-N) was very low (0.7-0.9 mg/l) before mixing with recycled effluent. The concentration increased to 12 and 25 mg/l in systems A and B respectively after mixing. Denitrification in the horizontal flow bed reduced NO$_x$-N to 0.3-0.5 mg/l and increased again by nitrification in the vertical flow bed to 38 and 73 mg/l in system A and B respectively.

The average concentration of COD in the raw wastewater was 1072 mg/l and 1243 mg/l in systems A and B respectively. The reduction was about 60% in horizontal flow bed and over 70% in the vertical flow bed resulting in 91-98% removal efficiency in the final effluent.

During November and February the hydraulic loading rate of raw wastewater plus recycled effluent was increased to 6 cm/d. The average temperature of the wastewater was 25°C and pH 7.9. TKN and NH$_3$-N of raw wastewater were around 414 and 346 mg/l and diluted to about 252 and 218 mg/l after mixing for system A, and 430 and 361 mg/l and diluted to 243 and 199 mg/l after mixing for system B, respectively. In this case, the removal efficiency of the systems were lower than the previous run because of the high density of plants was higher that previous run, resulting in higher nitrogen and carbon from plants debris in the effluent and the system was clogged the last few weeks. In the system B, the high evaporation and evapo-transpiration rate made that plants were not healthy and wilted. Therefore, the removal efficiency of TKN in systems A and B were reduced to 68 and 88% respectively. The transformation to oxidised nitrogen was satisfactory. NO$_x$-N in the mixed water was between 19.3 and 50.5 mg/l in both systems A and B. Denitrification in horizontal flow bed reduced NO$_x$-N to 0.1 and 1.2 mg/l and increased again to 47.5 and 98.4 mg/l in the vertical flow beds of system A and B respectively. The removal efficiency of BOD and SS was 90 to 99%.

Once the problems in the former campaign were solved, the last testing period started. For this period, the hydraulic loading rate was 12 cm/d. The average temperature and pH in the raw wastewater was 28°C and 7.8. TN removal efficiency was increased from 57 and 64% to 75 and 73% in system A and B, respectively. Other parameters had similar removal performances.

A performances summary of these systems is possible to see in the Table 3.9.

Kantawanichkul et al. (2002) concluded that both systems showed high efficiency of N, organic carbon, SS and TP removal. Denitrification was promising in saturated horizontal flow bed and nitrification performance was carried out in the vertical flow vegetated bed. The performances of system A and B did not show clear differences. It was also found that at low hydraulic loading rate (3 cm/d) in both systems gave higher removal efficiencies than 6 and 12 cm/d, especially in system B.
Table 3.9 Removal performances of both systems A and B depending on the loading rate. (Kantawanichkul et al., 2002)

<table>
<thead>
<tr>
<th></th>
<th>3 cm/d</th>
<th></th>
<th>6 cm/d</th>
<th></th>
<th>12 cm/d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw A</td>
<td>%</td>
<td>Raw B</td>
<td>%</td>
<td>Raw A</td>
<td>%</td>
</tr>
<tr>
<td>TN</td>
<td>411</td>
<td>76</td>
<td>416</td>
<td>79</td>
<td>415</td>
<td>57</td>
</tr>
<tr>
<td>TKN</td>
<td>411</td>
<td>85</td>
<td>415</td>
<td>98</td>
<td>414</td>
<td>68</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>328</td>
<td>84</td>
<td>346</td>
<td>98</td>
<td>346</td>
<td>66</td>
</tr>
<tr>
<td>NOₓ-N</td>
<td>0.7</td>
<td>-</td>
<td>0.9</td>
<td>-</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>COD</td>
<td>1072</td>
<td>91</td>
<td>1243</td>
<td>95</td>
<td>1195</td>
<td>86</td>
</tr>
<tr>
<td>SS</td>
<td>547</td>
<td>98</td>
<td>1023</td>
<td>99</td>
<td>1302</td>
<td>99</td>
</tr>
</tbody>
</table>

3.4 Phosphorus

Phosphorus, as nitrogen, is also essential to the growth of algae and other biological organisms. Because of noxious algal blooms that occur in surface water, efforts have been made to control the amount of phosphorus compounds that enter surface waters in domestic and industrial waste discharges and natural runoff. Municipal wastewater may contain from 4 to 12 mg/l of phosphorus as P (Crites and Tchobanoglous, 1998).

The phosphorus presence in raw wastewater may come from the following sources:

- The carriage water (usually minor).
- Faecal and waste materials.
- Industrial and commercial uses.
- Synthetic detergents and household cleaning products.

3.4.1. The phosphorus cycle

Phosphorus is typically present in wastewater as orthophosphate, dehydrated orthophosphate (polyphosphate) and organic phosphorus. Biological oxidation results in the conversion of most phosphorus to the orthophosphate forms (H₂PO₄⁻, HPO₄²⁻, PO₄³⁻). No changes in valency take place with phosphorus during biotic assimilation of inorganic P or during decomposition of organic P by microorganisms. Soil P primarily occurs in +5 (oxidized) valency state, because all lower oxidation states are thermodynamically unstable and are readily oxidized to PO₄³⁻ even in highly reduced wetlands soils. Phosphorus has a gaseous phase (phosphine) that has only been measured in ultratrace amounts (Vymazal et al., 1998).

Phosphorus removal in wetland treatment systems occurs from adsorption, plant absorption, complexation and precipitation reactions with aluminium (Al), iron (Fe), calcium (Ca) and clay minerals in the bed matrix (Cooper et al. 1996). Most wetland studies have shown that sediment/peat accumulation is the major long-term phosphorus sink and that wetlands are not particularly effective as phosphorus sink, when compared with terrestrial ecosystems. The sediment-litter compartment is the major P pool (over
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95%) in natural wetlands, with much lower plant pool and little in the overlying water (Vymazal et al., 1998).

Adsorption and retention of phosphorus in wetland soils is controlled by the interaction of redox potential, pH value, Fe, Al, Ca minerals and the amount of native soil P. In acid soils, inorganic P is adsorbed on hydrous oxides of Fe and Al and may precipitate as insoluble Fe-phosphates (Fe-P) and Al-P. Precipitation as insoluble Ca-P is the dominant transformation at pHs greater than 7.0. Adsorption of P is greater in mineral vs. organic soils. Inorganic soils P adsorption has been related to either high Al, Fe or Ca levels and P sorption capacity of wetlands soils may be predicted solely from the oxalate-extractable (amorphous) aluminium content of the soil. Redox potential below +250 mV will cause the reduction of Fe$^{3+}$ to Fe$^{2+}$, releasing associated P. On the other hand, the reduction of redox potential caused by flooding can cause the transformation of crystalline Al and Fe minerals to the amorphous forms; and amorphous Al and Fe hydrous oxides have higher P sorption capacity than crystalline oxides due to their larger number singly-coordinated surface hydroxyl ions. Faulkner & Richardson (1989) reported that the most important retention mechanisms are claimed to be ligand exchange reactions, where phosphate displaces water or hydroxyls from the surface of Fe and Al hydrous oxides to form monodentate and binuclear complexes within the coordination sphere of the hydrous oxide (Vymazal et al., 1998). Cooper et al. (1996) pointed out that it is yet unclear whether specific adsorption via ligand exchange or precipitation reaction is the major P removal mechanism in constructed wetlands. Some results show rapid P removal from solution, indicating an adsorption mechanism. However, this fast removal is often followed by a much slower process giving rise to a less exchangeable form. This slow reaction has been assumed to be precipitation of insoluble phosphates or conversion from monodentate to binuclear complexes or both.

Phosphate precipitation may also be achieved by the addition of the salts of one of three metals that form sparingly soluble phosphates. These are calcium (Ca(II)), iron (either ferric ion, Fe(III), or ferrous ion, Fe(II)) and aluminium (Al(III)). The salts most commonly employed are lime (Ca(OH)$_2$), alum (Al$_2$(SO$_4$)$_3$?18 H$_2$O), sodium aluminate (NaAlO$_2$), ferric chloride (FeCl$_3$), ferric sulphate (Fe$_2$(SO$_4$)$_3$), ferrous sulphate (FeSO$_4$) and ferrous chloride (FeCl$_2$). Knowledge of the nature of the phosphates formed by addition of these precipitants to wastewater and of their solubilities and the variation of solubilities with solution conditions is essential for predicting and controlling the results of chemical phosphate removal (Sedlak, 1991).

On the other hand, plant absorption consists of plants absorbing phosphorus through their roots and transport it to the growing tissues. The uptake capacity of macrophytes is lower as compared to nitrogen as phosphorus concentrations in plant tissues are much lower than that of nitrogen (Brix, 1994; Vymazal, 1995). In order to remove phosphorus from wetlands in this way it is necessary to harvest macrophytes biomass; this is especially necessary for free-floating plants. After the plant decay, similarly to nitrogen, phosphorus is released back to the system. In addition the amount of phosphorus removed by emergent vegetation forms only a small fraction of the local phosphorus removed in a constructed wetland (Vymazal et al., 1998).
Furthermore, Brix (1998) and Vymazal \textit{et al.} (1998) pointed out that subsurface flow constructed wetland systems treating domestic sewage are known to be poor in removal of phosphorus. Plant uptake can be a sustainable removal mechanism if it is subsequently harvested. However, the amount of phosphorus that can be removed by harvesting the plants usually constitutes only a small fraction of the amount of phosphorus loaded into the system with the sewage. Phosphorus may also be bound to the media of the reed beds mainly as a consequence of adsorption and precipitation reactions with calcium (Ca), aluminium (Al) and iron (Fe) in the sand or gravel substrate. The capacity of a reed bed to remove P may therefore be dependent on the contents of these minerals in the substrate. This hypothesis is supported by the observation that P-removal has been found to be particularly efficient in constructed reed beds containing ferruginous sand. However, the P removal efficiency is often high initially and then decreases after some time as the P sorption capacity of the sand is used up. It is therefore of paramount importance to select a sand medium with a high P-sorption capacity in order to obtain a sustained P-removal in the long term constructed reed bed system (Arias \textit{et al.}, 2001). Some experiments were carried out in the laboratory of Plant Ecology, Aarhus University, Denmark, in order to determine the highest P-removal media in Denmark (see Arias \textit{et al.}, 2001).

3.4.2. A removal phosphorus solution

At the Plant Ecology laboratory of the University of Århus, some removal studies about phosphorus have been carried out, a summary of a paper by Arias \textit{et al.} (2002) is presented:

\textit{Phosphorus removal from municipal wastewater in an experimental two-stage vertical flow constructed wetland system equipped with a calcite filter} (Arias \textit{et al.}, 2002).

To solve the problem about phosphorus removal it is necessary to select a medium with a high P-binding capacity, but even if this is done the binding capacity will be used up after a few years and something have to be done to sustain phosphorus removal. Replacing the bed medium will be far too expensive. A sustainable solution would be to establish a separate filter unit containing material with a high P-binding capacity, and to replace the material at a regular basis.

After some laboratory studies a calcite product was identified with potential for high P-binding capacity, as well as good hydraulic and physical characteristics. The studies showed that about 30 kg of calcite per PE per year would be needed to bind phosphorus. Based on these studies a full-scale experimental constructed wetland was built with a calcite-based filter unit included in the flow path in order to study the performance of the filter under real-life operational conditions.

The two-stage experimental vertical flow constructed wetland system consist of a three chamber $2 \text{ m}^2$ sedimentation tank, two vertical flow beds and three filter-units in series containing calcite and with vertical upflow to study the removal of phosphorus. The filters are constructed in 40 cm diameter wells (filter depth 70 cm) (see Figure 4.2). This experimental plant is the same one that it has been used to develop the current paper.
Eight runs were carried out at different times of the year. The campaigns were carried out in pairs, with and without recycling the effluent to the sedimentation tank. In the campaign 1 and 2 the P-filters were empty. In all campaigns a loading rate of 2 m$^3$/d of raw sewage into the sedimentation tank was targeted. However, particularly during campaign 4, 7 and 8 some heavy precipitation occurred and increased the hydraulic loading rate of the system. The targeted recycling was 100%. The hydraulic loading rate of the P-filters was very high, 13-50 m/day. Hence the nominal residence time of the water in the calcite filters ranged between 28 and 99 minutes.

The removal rate in the sedimentation tank averaged 30 ± 18% of the phosphorus mass loading (16.6 ± 8.3 mg/l). The phosphorus removal in the first constructed wetland was 24 ± 12% of the loading (average 6.5 ± 1.9 gP/m$^2$/day). For campaign 1 and 2 the first bed was the 10 m$^2$, and for the remaining campaign the 5 m$^2$ bed.

The calcite in P-filters was installed before campaign 3. The P-filters removes phosphorus very effectively during campaign 3, decreasing the P concentration by 75% from 5.7 ± 0.5 mg/l in the inlet of the filters to 1.4 ± 0.6 mg/l in the effluent from the third filter unit (see Figure 3.19). During the following campaigns the removal performance of the filters decreased. Already at campaign 4, which was conducted 7 weeks after the P-filters were installed, the first P-filter seemed to be saturated by phosphorus, and so did not bind any P. However, the second and in particular the third P-filter, still removed significant amounts of P. During the last three campaigns, the calcite filters seemed to be P-saturated, and they do not remove very much P. The total amount of phosphorus removed by the P-filters during the operation of the system was about 600 g of P in total, which equivalent to 2.2 kg/m$^3$. The effluent from the P-filters were loaded onto the second vertical bed where the percentage of removal was no significant.

Therefore, the overall removal efficiency of the two-stage experimental system ranged between 30 and 87% (average 62 ± 18%). The lowest removals were recorded at campaign 7 and 8, which had very high hydraulic loading, rates and dilute wastewater.

Arias et al. (2002) pointed out the removal percentage in the P-filter was lower than expected in the P-filters. The reason could be a short hydraulic retention time, or the contact time, in the filter. The earlier studies in the laboratory showed a high removal percentage because the hydraulic retention time was of 10 to 12 hours while in the full scale P-filters the time range was only between 28 to 99 minutes.
Figure 3.19 (A) Total-P in the three P-filters inlet and in the outlet of each one. (B) Phosphorus removal in the P-filters inlet and in the outlet of each one (Arias et al., 2002).

Based on the average inlet and outlet concentration of total-P and hydraulic loading rates of each individual bed the area based first order removal constants were estimated. The results showed that the removal rate increased with loading rate. The area based rate constant calculated from the data was no constant and seem to depend on the mass loading rate, suggesting that the first order removal kinetic does not describe removal of phosphorus in vertical flow constructed wetlands.