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Treatment of domestic wastewater using microbiological processes and hydroponics in Sweden

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ABSTRACT

Conventional end-of-pipe solutions for wastewater treatment have been criticized from a sustainable view-point, in particular regarding recycling of nutrients. The integration of hydroponic cultivation into a wastewater treatment system has been proposed as an ecological alternative, where nutrients can be removed from the wastewater through plant uptake; however, cultivation of plants in a temperate climate, such as Sweden, implies that additional energy is needed during the colder and darker period. Thus, treatment capacity, additional energy usage and potential value of products are important aspects considering the applicability of hydroponic wastewater treatment in Sweden.

To enable the investigation of hydroponic wastewater treatment, a pilot plant was constructed in a greenhouse located at Överjärva gård, Solna, Sweden. The pilot plant consisted of several steps, including conventional biological processes, hydroponics, algal treatment and sand filters. The system treated around 0.56-0.85 m^3 domestic wastewater from the Överjärva gård area per day. The experimental protocol, performed in an average of twice per week over a period of three years, included analysis and measurements of water quality and physical parameters. In addition, two studies were performed when daily samples were analysed during a period of two-three weeks. Furthermore, the removal of pathogens in the system, and the microbial composition in the first hydroponic tank were investigated.

Inflow concentrations were in an average of around 475 mg COD/L, 100 mg Tot-N/L and 12 mg Tot-P/L. The results show that 85-90% of COD was removed in the system. Complete nitrification was achieved in the hydroponic tanks. Denitrification, by means of pre-denitrification, occurred in the first anoxic tank. With a recycle ratio of 2.26, the achieved nitrogen removal in the system was around 72%. Approximately 4% of the removed amount of nitrogen was credited to plant uptake during the active growth period. Phosphorus was removed by adsorption in the anoxic tank and sand filters, natural chemical precipitation in the algal step induced by the high pH, and assimilation in plants, bacteria and algae. The main removal occurred in the algal step. In total, 47% of the amount of phosphorus was removed. Significant recycling of nitrogen and phosphorus through harvested biomass has not been shown. The indicators analysed for pathogen removal showed an achieved effluent quality comparable to, or better than, for conventional secondary treatment. The microbial composition was comparable to other nitrifying biological systems. The most abundant phyla were *Betaproteobacteria* and *Planctomycetes*.

In Sweden, a hydroponic system is restricted to greenhouse applications, and the necessary amount of additional energy is related to geographic location. In conclusion, hydroponic systems are not recommended too far north, unless products are identified that will justify the increased energy usage. The potential for hydroponic treatment systems in Sweden lies in small decentralized systems where the greenness of the system and the possible products are considered as advantages for the users.

Key words: biological, hydroponics, microbial composition, nitrogen, organic matter, phosphorus, small scale, theoretical energy requirements, wastewater treatment.

SAMMANFATTNING

Konventionella "slut på röret" lösningar för avloppsvattenrening har tidigare blivit kritiserade ur ett uthållighetsperspektiv, i synnerhet med avseende på återanvändning av näringsämnen. Ett föreslaget ekologiskt alternativ är att integrera hydroponisk odling med avloppsvattenrening, och därmed återanvända näringsämnena i form av gröna växter eller andra produkter. För odling av gröna växter i temperat klimat kommer extra energi att krävas under årets mörka och kalla period. Därför är reningskapacitet, såväl som extra energiåtgång och potentiellt värde hos de produkter som framställs ur systemet, viktiga aspekter när det gäller lämpligheten för hydroponisk avloppsvattenrening i Sverige.

För att möjliggöra en undersökning av hydroponisk avloppsvattenrening i Sverige, har en pilotanläggning byggts i ett växthus på Överjärva gård i Solna. Pilot-anläggningen var uppbyggd av flera steg, och innefattade konventionella biologiska processer, hydroponiska tankar, algsteg och sandfilter. Systemet renade 0.56-0.85 m^3 hushållsspillvatten per dag. I genomsnitt, har analyser och mätningar av vattenkvalitets-parametrar och fysiska parametrar utförts varannan dag under en tre-års period. Två intensivstudier har utförts, Juli 2002 och Februari 2003, där dagliga analyser gjordes under en period av två till tre veckor. Vidare har systemets reduktion av patogena mikroorganismer och den mikrobiella sammansättningen i den första hydroponiska tanken undersökts.

Medelvärdet för koncentrationerna i inflödet var runt 475 mg COD/ml, 100 mg Tot-N/L och 12 mg Tot-P/L. Resultaten visar att 85-90% av COD renades i systemet. Fullständig nitrifikation uppnåddes i de hydroponiska tankarna. Systemet var konstruerat med för-denitrifikation, och med en recirkulationsgrad på 2,26 erhölls 72% rening av kväve i systemet. Ungefär 4% av den bortförda kvävemängden tillskrevs växtupptag under sommarsäsongen. De processer som bidrog till fosforreduktionen var adsorption i den anoxiska tanken och sandfiltren, naturlig kemisk fällning framkallad av högt pH i algtankarna, och assimilering hos växter, bakterier och alger. Huvuddelen av fosforreningen skedde i algtankarna. Totalt uppnåddes 47% rening av fosfor i systemet. Betydande återanvändning av kväve och fosfor i form av skördad biomassa har inte kunnat påvisas. Analyser av indikatororganismer för patogener visade att kvalitén på utflödet motsvarade, eller var bättre än, för konventionell biologisk rening. Den mikrobiella sammansättningen var jämförbar med andra biologiska nitrifierande system, och de mest förekommande fyla var *Betaproteobacteria* and *Planctomycetes*.

I Sverige är hydroponisk avloppsvattenrening begränsad till växthusmiljö, och behovet av extra energi för värme och ljus är relaterat till den geografiska placeringen. På grund av den ökade energiförbrukning, rekommenderas dessa system för nordliga breddgrader under förutsättning att produkter identifieras som uppväger energiåtgången. Potentialen för hydroponisk avloppsvattenrening ligger i små decentraliserade system där systemets grönska och möjliga produkter kan anses vara en tillgång för systemets användare.

List of Papers

This thesis is based on the following original research papers, which in the text will be referred to by their roman numerals.

I. A small scale hydroponics wastewater treatment system under Swedish conditions. **Norström, A.**, K. Larsdotter, L. Gumaelius, J. la Cour Jansen and G. Dalhammar (2003). Water Science and Technology, 48(11-12): 161-167.

II. Removal of microorganisms in a small-scale hydroponics wastewater treatment system. Ottoson, J., **A. Norström** and G. Dalhammar. Letters in Applied Microbiology, *in press.*

III. Theoretical energy requirements for maintenance of green plants in hydroponic wastewater treatment. Larsdotter K., **A. Norström** and G. Dalhammar (2004). Vatten, 60: 187-191.

IV. A comparative study of six hydroponic wastewater treatment plants. **Norström, A.** (2005). Vatten, 61(2): xx-xx.

V. Energy savings versus year-round production in a small hydroponic system for wastewater treatment. **Norström, A.**, J. la Cour Jansen and G. Dalhammar. *Accepted for publication in Water Science and Technology.*

VI. Investigation of the microbial composition in the hydroponic step of a treatment system for domestic wastewater with fluorescent *in situ* hybridization (FISH). **Norström, A.**, G. Dalhammar and N. Lee. *Submitted to Ecological Engineering.*

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INTRODUCTION

One hundred years of urban wastewater treatment

At the turn of the 20th century in Sweden, wastewater treatment was almost non-existent and simple ditch or pipe systems transported the raw wastewater directly to the receiving waters. Around this time the water closet (WC) was more commonly introduced, although highly opposed by the city authorities due to fear of clogging sewers and nuisance in the receiving waters. Some cities permitted the installations under condition that the house-owners inserted a cess-pool for storage or a septic tank before discharge to the public sewer. The first treatment works, a primitive trickling filter, was built in 1911 in Skara, although it had few successors. It was rather through the arrival of a German invention in 1906 that the first step towards common improved treatment started. The Imhoff tank was mainly a modified septic tank where the liquid was separated from the anaerobic digestion of the solids, thereby avoiding the anoxic conditions. These tanks remained the dominating treatment technique until the mid 1930s (Isgård, 1998).

In spite of the cities' resistance towards the WC, their numbers increased. Besides being a modern invention, thus desirable, this was partly due to the existing nuisance with the dry toilets, such as smell and problems with collection and treatment of the latrine. Later, however, there were also drawbacks with the required septic tanks. They were not emptied regularly and the control of the system was difficult. In 1935, WCs were allowed in all Swedish cities besides Borgholm and Mariefred. There were already 270,000 WCs in the three largest cities alone, however, statistics show that only 11 out of 111 Swedish cities treated their wastewater (Isgård, 1998). By now, the increasing negative effects on the recipients were obvious, severe pollution of water reservoirs and bathing areas, and reports of fish kill were common. In 1928 an investigation concerning the water conditions in Stockholm started. The conclusion was a need for primary treatment, i.e. removal of floating and suspended solids, before discharge to the recipient (Cronström, 1986). As a result, several treatment works were constructed around this time. Among them was the largest wastewater treatment plant (WWTP) in Sweden, Henriksdalsverket, inaugurated in 1941. In the original set-up, the treatment at Henriksdal WWTP included screening, grit removal, grease collectors, sedimentation tanks and digesters (Isgård, 1998).

During the second world war the interest for improving wastewater treatment declined. Therefore in 1950, around 90% of the population in the cities still had no treatment of their wastewater. In total, there were only four works with biological treatment through trickling filters and a couple of activated sludge plants. In 1953 the big wake-up call arrived in the form of an epidemic of paratyphus; and it became evident that the cities had to address the question of pollution from untreated or poorly treated wastewater from a hygienic view-point. From now on the application of biological treatment, also denoted secondary treatment, really started. In nature, oxidation of organic matter as a form of energy source is performed by aerobic heterotrophic bacteria, and this process demands oxygen. Usually, the concentration of organic matter is high in wastewater, i.e. the biochemical oxygen demand (BOD) is high, even after primary treatment. If wastewater is discharged untreated, the biological processes will result in depletion of the receiving water's oxygen supply, with subsequent negative effects on aquatic life. In biological treatment, heterotrophic bacteria are used in a confined aerated environment to remove BOD from the effluent. The bacteria also utilize organic matter for growth, hence the production of biomass or sludge in a biological treatment step.

At this time, the technical developments concerning wastewater treatment were mainly concentrated on the activated sludge process, at least for larger treatment plants. The activated sludge process was incorporated into Swedish treatment plants, and soon became the dominating treatment technique. In parallel, another American method was applied for smaller communities of less than 3000 people. The stabilization ponds, or lagoons, had been developed in cold states and were considered applicable for BOD removal even with around 1-3 months of frozen ponds. Foremost, the costs for construction and maintenance were low. The first large system was built on Gotland in 1954, and by 1970 the 128 constructed lagoons were by numbers the most common treatment technique in Sweden. In 1966, WCs were installed in 95% of the city-buildings. Four years later, 5.3 million people or 86% of the town-residents, were included in the municipal wastewater treatment. Statistics show that 70% were connected to secondary treatment (activated sludge 56.5%, trickling filters 10.4%, stabilization ponds 3.0%); while the rest had primary treatment (28.6%) or treatment of an undefined type (Isgård, 1998).

During the 1960s, nutrient removal appeared strongly on the agenda. This was mainly due to the fact that man-made eutrophication was causing an obvious increasing and undesirable degradation of water quality in lakes and reservoirs. Eutrophication is a result of increased input of nutrients, mainly phosphorus and nitrogen, to a water body. The nutrient rich environment causes increased primary production, i.e. growth of algae. As a results of eutrophication, great physical, chemical and biological changes occur in an aquatic ecosystems, such as decreased light penetration, oxygen deficiency and fill kill (Forsberg, 1991). Thus, the next cycle in wastewater treatment emerged, the introduction of tertiary treatment, i.e. reduction of nutrients.

In Sweden phosphorus was identified as the causing nutrient of eutrophication in our lakes and streams. Moreover, basic knowledge of the chemical precipitation process was already at hand, and thus the introduction was relatively fast. By 1970 there were already 16 WWTP with chemical precipitation in Sweden, and during the next two decades it was implemented on the large scale. Contributing factors to the fast expansion of WWTPs with chemical precipitation in Sweden were the availability of inexpensive precipitants and perhaps mainly the incentive state grants. For solely biological treatment, the expansion grants were 35%; whereas for a combined biological and chemical plant the grants were 50% (Isgård, 1998).

During the 1980s, extensive algae blooms effecting Swedish coastal areas occurred. This time, nitrogen was considered a significant factor. As a result, additional effluent restrictions concerning the nitrogen discharge, especially to coastal waters, were made. These restrictions laid the foundation for the expansion of nitrogen treatment during the 1990s (Isgård, 1998). Biological treatment for nitrogen is a combination of two separate microbial processes, nitrification and denitrification. In wastewater, nitrogen appears mostly in the form of urea, ammonium ions or bound in organic material, which after decomposition in the biological treatment are transferred to mainly inorganic ammonium ions. During nitrification, ammonium is oxidized to nitrate by a few specific groups of bacteria in an aerobic environment. Nitrifying bacteria are characterized by a low growth rate, since the oxidation of ammonium and nitrite gives a low energy yield. Most of the nitrifyers are autotrophs, which means that they use carbon dioxide as carbon source. In the proceeding anoxic environment nitrate is used instead of oxygen in the respiration process, thereby providing the bacteria with energy. The anoxic environment is essential since most denitrifyers are facultative bacteria, and therefore would use oxygen, which gives a higher energy yield instead of nitrate, if possible. During the denitrification process, nitrate is transformed to nitrogen gas and thus removed from the aquatic phase.

Today there are around 500 conventional wastewater treatment plants with a licence duty, i.e. designed for more than 2000 population equivalents (p.e.) in Sweden. Among these plants around 200 have the capacity to treat wastewater from more than 10,000 p.e. (SNV, 2003). Since the 1990s these plants have, to a large extent, been further developed for special nitrogen treatment. Almost all urban households are now connected to the municipal treatment, and around 95% of the urban wastewater is treated both chemically and biologically. In Figure 1, the development of the treatment in the 500 municipal treatment plants is shown by percentage of the urban population (SNV, 2003).

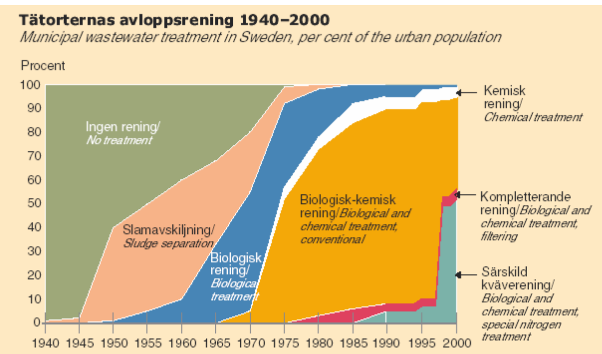

Figure 1. Municipal wastewater treatment in Sweden (SNV, 2003).

On-site systems

According to the historical story above, it seems that wastewater treatment in Swedish urban areas is a problem of the past, however, outside the urban areas there are an estimated one million households that are not connected to the municipal treatment plants. Wastewater from these households are mainly treated by various kinds of on-site systems; although it is believed that less than 50% of the systems fulfill the legal requirements of performing more effective treatment than sludge removal alone (SNV, 2002a). The nutrient discharge, in particular phosphorus, from these scattered dwellings are considered to be larger than the discharge from the municipal treatment plants. During the period, 1985-1999, a total of 650 tonnes per year, i.e. 21% of the anthropogenic phosphorus gross load on the Swedish seas, was accounted to scattered dwellings. For comparison, the discharge from wastewater treatment plants was 16%. During the same period in average 3%, 2700 tonnes per year, of the total anthropogenic nitrogen net load to the coastal waters were accounted to scattered dwellings, as compared to a 21% contribution by the treatment plants. Although implemented improved treatment techniques will decrease these numbers, it is interesting to note that the largest contributions of both anthropogenic nitrogen (58%) and phosphorus (46%) were from agriculture (SNV, 2002b).

Today there are many possibilities for treatment of scattered dwellings; and studies to compile and compare available alternatives have been made (e.g. SNV, 2002a; af Petersens, 2003). In general, these on-site systems can be divided into source separating systems and end-of-pipe solutions. The first group includes source separation of urine, black water and dry systems. Most aspects of urine separation has been well studied, and current know-how and experiences can be found in several reports (e.g. Byggforskningsrådet, 2000). Even with a separating system, however, there will always be a remaining effluent that needs treatment.

Examples of current end-of-pipe solutions are:

- small treatment plants based on biofilm processes (biorotors, trickling filters or submerged biobeds)
- small treatment plants based on the sequencing batch reactor (SBR) technology
- complementary chemical precipitation
- artificial filters
- "natural systems" (e.g. reed beds, infiltration units, sand filters)

Results from different on-site systems, treating between 1-500 persons, has previously been compared to evaluate the different alternatives (SNV, 2002a). Small plants based on biofilm processes appeared more flexible regarding large variations in hydraulic and organic load than activated sludge processes, i.e. SBR reactors. Although the SBR technology was also considered robust, with generally good experiences reported. Many of the small plants used chemical precipitation with good results; although inadequate dosing equipment could be a problem. The use of materials with high phosphorus absorption in artificial filters were considered to be the least explored area with many unresolved questions. These questions relate to performance (practical function, hydraulic performance, sorption ability) and reuse issues such as: plant availability of the sorbed phosphorus, amount of contaminants and handling and spreading of the potential fertilizers (SNV, 2002a). Complementary chemical precipitation was usually used as pre-treatment for infiltration and sand filter units. The results were promising; although the risk for clogging needs to be further evaluated (SNV, 2002a). Presently sand filters and infiltration units are the most common on-site treatment systems in Sweden for single households. These and other natural systems will be further explained in the next chapter.

Natural systems

Natural "green" systems are often intended to describe processes that depend primarily on their natural components to achieve the intended purpose. They might typically include pumps and piping for waste conveyance but would not depend exclusively on external energy sources to maintain the major treatment responses (Reed et al., 1995). Natural systems for effective wastewater treatment are available in three major categories: terrestrial, wetland and aquatic concepts (Reed et al., 1995; Tchobanoglous, 1997).

Terrestrial systems

The terrestrial systems are divided into three basic concepts: slow-rate irrigation, rapid infiltration, and overland flow. Overland flow systems, where water is allowed to percolate through a thin litter layer over a sloping planted surface, is not used in Sweden (Wittgren and Hasselgren, 1992). The other two systems are characterized by a subsurface vertical flow. Crop irrigation demands a relatively large area and can be used for fast growing energy crops such as *Salix* spp. Infiltration units are the most commonly recommended and used treatment method for treatment of wastewater from single households in Sweden (SNV, 1997; 2002a). In infiltration units, the effluent from septic tanks percolates through a buried bed consisting of a nutrient poor substrate, such as washed macadam, where degradation of organic matter takes place. Phosphorus is adsorbed in the soil layer, and some removal of nitrogen occurs. Construction of an infiltration unit is dependent on the land profile and characteristics of the soil. If the natural soil is unsuitable, then sand filters can be used. Sand filters resemble infiltration units, but usually have two spreading layers and an additional layer of sand, and therefore a defined limited volume for filtration (SNV, 1998). Vegetation should be avoided since the root systems might clog the distribution pipes (SNV, 1997). A combination between infiltration units and reed beds is infiltration wetlands, which are planted and the water is spread on the surface instead of under ground (Wittgren and Hasselgren, 1992).

Wetland systems

Wetlands are defined as land where the water table is at, or above, the ground surface long enough each year to maintain saturated soil conditions and growth of related vegetation (Reed et al., 1995). The type of wetland used is determined by the required treatment, i.e. secondary of tertiary, mass loading, climate, available land and cost (Greenway, 2003). There are two main categories of constructed wetlands:

1. *Freewater surface flow wetlands* resemble natural wetlands, and are composed of shallow vegetated channels or basins, often referred to as marshes, and deeper open water ponds. They are typically used to provide tertiary treatment after conventional secondary treatment, which removes most of the organic pollutants.

2. *Subsurface flow wetlands*, also called reed beds or root-zone systems, are gravel, sand, or soil filled channels, or basins with no standing water. They are typically used by small communities or single households for secondary treatment after screening and primary treatment.

Compared to stabilization ponds, emergent vegetation is the dominant feature of constructed wetlands. The plants serve multiple purposes in wastewater treatment wetlands, and the selection of plant species depends upon the local availability of species, the type of wetland and the chemical composition of the wastewater effluent (Greenway, 2003). Stems, leaves and roots act as filters for suspended solids, reduces water current velocity and thereby promotes sedimentation of particles. The vegetation also provides an increased surface area for microorganisms active in the biological processes, and can thus be regarded as a huge biofilm. Oxygen leakage from roots creates oxidized conditions which stimulates both aerobic decomposition of organic matter and growth of nitrifying bacteria in the rhizosphere (Brix, 1994; Greenway, 2003). The relative importance of plant oxygen transfer for treatment in wetlands is, however, considered low (Brix, 1994), and anaerobic conditions will predominate unless the organic load is low, the wetland is shallow enough, or aeration devices are incorporated (Wood, 1995). Inorganic nitrogen and phosphorus are essential for plant growth, thus removal is achieved through plant uptake. As wetland plants are very productive, considerable amount of nutrients can be bound in the biomass. However, compared to the nutrient loading with the wastewater into the wetland, Brix (1994) states that the amount of nutrients removed through uptake is insignificant. For example, if a wetland with water hyacinths, a floating aquatic plant that generally have a high uptake capacity, was to rely exclusively on macrophyte uptake for nutrient removal, an area of 30-50 m^2 per p.e. is needed (Brix, 1994). Plants also have additional functions that are not related to wastewater treatment, which may be significant at specific sites. These functions include odor control, insect control, support of a diverse wildlife and aesthetic value (Brix, 1994; Wood, 1995).

There are several advantages with the subsurface flow concept. Since the gravel media provide most of the surface area for the biological reactions, water passing the gravel bed will have higher reaction rates. The water surface is below the top of the media, which prevents mosquito and public access problems. This characteristic also makes them more suitable for cold climates since the microbial processes can still occur in the root-zone during winter (Reed et al., 1995; Greenway, 2003). Mæhlum and Jenssen (2003) concluded that subsurface wetlands could be applied for improvement of water quality parameters in cold climate if there is efficient aerobic pre-treatment, low hydraulic loading rates, a specific filter media and adequate insulation. However, relatively high costs are associated with media supply and construction, and maintenance if clogging occurs. In conclusion, a subsurface system requires approximately one quarter of the surface area required for a freewater surface flow system, and they cost approximately four times as much. (Reed et al., 1995). In Sweden, subsurface wetlands are not commonly used (Wittgren and Hasselgren, 1992).

Aquatic systems

Aquatic systems are further subdivided to distinguish between two categories. Lagoon and pond systems depend on microbial life and the lower forms of plants and animals, whereas aquatic treatment systems also utilise higher plants and animals (Reed et al., 1995). Stabilization ponds are usually large, and the three main types are classified by the dominant biological reaction in the ponds.

1. *Anaerobic ponds* are usually used as a pre-treatment step for sewage with a high concentration of solids, and for treatment of strong industrial and agricultural wastes. Due to heavy organic loading, there is no aerobic zone and the principal biological reactions are acid formation and methane fermentation in the sediment (Reed et al., 1995). The remaining liquid is normally transferred to facultative ponds for posterior treatment.

2. *Facultative ponds* are the most frequently used stabilization ponds. The key to facultative operation is oxygen production by photosynthetic algae and oxygen transfer at the surface. Anaerobic fermentation occurs in the lower layer and aerobic stabilization in the upper layer. One of the most serious performance problems of facultative ponds is the presence of algae in the final effluent (Reed et al., 1995).

3. *Aerobic ponds* are usually shallow to allow light to penetrate and to maintain dissolved oxygen throughout their entire depth. They are common as treatment after facultative ponds, where the main function is to destroy pathogenic organisms. Aerated ponds, by mechanical or diffused aeration, are usually deeper, with a longer detention time (Reed et al., 1995), and used for degradation of organic matter.

An aquatic treatment system is defined as the use of aquatic plants or animals as a component in a wastewater treatment system. The major biological components include floating plants, fish and other animals, planktonic organisms and submerged plants. The treatment responses are due either to the direct uptake of material by the plants or animals, and by the presence of these biota altering the physical environment in the system. In addition the vegetation act as host substrate for attached microbial organisms, which provide a very significant degree of treatment (Reed et al., 1995).

Hydroponics

In the 19th century two scientists, J. von Sachs and W. Knop, independently showed that terrestrial plants could grow in nutrient solution without soil. They demonstrated that plants could supply all their needs from inorganic elements and sunlight only. Today the technique to grow plants with roots emerged in solution is called hydroponics (Stanhill and Enoch, 1999). In literature, cultivation with a defined nutrient solution, i.e. hydroponics, is sometimes differentiated from cultivation with a natural solution, such as wastewater, which is called bioponics or organic hydroponics. In this thesis, the general term hydroponics is used as a definition for the technique to grow plants with roots emerged in wastewater. In contrast to conventional wastewater treatment systems, hydroponic systems are associated with greenness, since the reactors are completely covered with green plants (Figure 2).

Figure 2. The Överjärva pilot plant, March 2002.

One reason to introduce plants in wastewater treatment, is the possibility to recycle nutrients by means of production of valuable crops. Plants take up nutrients and water by their roots, hence in hydroponic wastewater treatment the wastewater can be regarded as a nutrient solution. In a soil substrate, the roots usually obtain sufficient oxygen; but when plants are grown in a liquid medium they might experience diffusion limitations. Oxygen deficiency inhibits cell respiration, which prevents an adequate supply of metabolic energy to drive absorption processes (Taiz and Zeiger, 1991). As a result, nutrient absorption and biomass production are reduced. Therefore, aeration of the fertilizer bulk solution in hydroponics is necessary to keep oxygen levels high in the vicinity of the roots. Other important parameters in hydroponics are pH and the nutrient concentration. The uptake rate changes according to the growth stage, the growing conditions and the ion source. Theoretically, the composition of the nutrient solution should be changed continuously, in order to be adjusted to the plant uptake (Stanhill and Enoch, 1999).

Vascular plants are macrophytes with internal vessels that can carry fluids. The fact that vascular plants can absorb, translocate, and metabolise or concentrate various chemicals has been known since the 1930's. Wolverton et al. (1976) stated that a controlled biological system, including a regular program of harvesting to achieve maximum growth and pollution removal efficiency, may represent an efficient and inexpensive filtration and disposal system for toxic materials and sewage released into waters near urban and industrial areas. Vascular plants passively or actively secrete photo-synthetically fixed carbon in forms susceptible to microbial degradation. The physiological conditions induced by the roots tend to be a stabilising factor in comparison with conditions created by bacteria alone in a mixed volume of activated sludge. Berndtsson (2001) thus argued that water hyacinth treatment systems are more stable than activated sludge systems. When hydroponics are used for wastewater treatment, the root system is also regarded as part of the microbial treatment process, since it provides a solid substrate for the microorganisms. A large root system is thus regarded as a positive property of the plants. In general, plant roots grow continuously; but their proliferation depends on the availability of water and minerals in their microenvironment. In regions that are poor in nutrients, proliferation is inhibited and the root system may die back. The surface area of the roots are greatly increased by root hairs (Figure 3), in some plants up to 60% of the surface area constitutes of root hair (Taiz and Zeiger, 1991).

Figure 3. The root system of Water Hyacinth (*Eicchornia crassipies*). Photo by M. Eriksson.

Sustainable Water Management

Until recently wastewater treatment has primarily concerned traditionally observed pollution effects such as oxygen depletion and eutrophication in receiving waters. The conventional end-of-pipe solutions for wastewater treatment have, however, been criticized from a sustainable view-point, in particular regarding recycling of nutrients. Fixated nitrogen is denitrified back to the atmosphere and phosphorus is bound in sludge, which today is no longer accepted as a fertilizer source by Swedish farmers. The importance of recycling phosphorus, which is considered to be a limited resource, has been thoroughly discussed and several methods to extract precipitated phosphorus from sludge are under investigation. Although nitrogen is not a limited resource, the nitrogen fixation process is costly and therefore recycling of nitrogen is also of interest. The possibility of using urine directly as a fertilizer source was one of the driving forces behind the development of urine separation systems. However, the main problem with residual sludge from wastewater treatment is not necessarily the sludge itself, but rather the high concentrations of heavy metals and toxic or other unwanted substances that comes with it. Wastewater contains substances such as pharmaceuticals, a great variety of household chemicals and substances discharged from trade and industry (Kroiss, 2004). It is fair to say, that the contents of sludge only reflects what the users of the system put into the system. It has been discussed that one of the reasons why toilets and sinks are considered to be the ultimate receivers of all possible kinds of waste is the lack of transparency in the urban treatment systems. Today, treatment is performed far away from the users of the systems, which has resulted in a system where my waste is somebody else's problem. This makes the treatment systems more complex than just technical solutions.

One way of addressing the problem with unwanted substances in the wastewater is to bring the treatment closer to the source by constructing decentralized systems. Another alternative is, to construct transparent systems and involve the users of the system. A combination of the two might be decentralized, transparent systems that utilizes the nutrients in the wastewater, i.e. production systems where nutrients are transformed into useful biomass as part of the treatment. Potential biomass production systems are the natural systems, which are also sometimes denoted green systems. Green often means that a technology requires less non-renewable energy sources than other alternatives. Although it should be noted, that for natural systems, "green" might be just a reflection of the growing vegetation, and not necessarily implying a more sustainable system (Brix, 1999).

Green systems might also be synonymous with ecological systems, although not necessarily in the form of large natural systems. Within the rather new discipline, Ecological Engineering, emphasis is on combining ecosystem function with human needs (Mitsch, 1997). Since the late 1980s, the term ecological engineering has been applied to the treatment of wastewater in ecologically based "green or living machines" with indoor greenhouse applications. These applications have been described as an environmentally responsible technology which would provide little or no sludge, generate useful byproducts, use no hazardous chemicals in the process chain and remove synthetic chemicals from the wastewater (Guterstam and Todd, 1990). A "living machine" is composed of a combination of steps which typically include settling tanks, aerated hydroponic tanks, clarifiers, "ecological fluidised beds" and gravel based wetlands (Todd and Josephson, 1996). They might also include a constructed aquatic food chain, based on algae, zooplankton and fish, and polishing steps using hydroponic cultivation (Guterstam, 1996). Although these systems have been tried and studied before, their actual contribution to increased sustainability remains to be discussed.

The assumption that wastewater treatment systems can no longer be regarded as just a matter of technical solutions, makes the selection of applicable treatment systems even more complex. Within the research program, *Sustainable Urban Water Management,* a common conceptual framework has been presented (Urban Water 2002), where the definition of a system included the technical structure as well as the organisation and users of the system. The users use the water and need to get rid of waste products. The technical system (e.g. pipes, pumps, treatment plants) supply the water and take care of the wastewater. The organisation owns, plans, finances, and manages the urban water system, and may be public or private, central or local.

This urban water system has then been analysed from five main groups of criteria or sustainability aspects, by means of several indicators and assessment tools. The five criteria considered to be most important were: health, environment, economy, socio-culture and technical function (Figure 4).

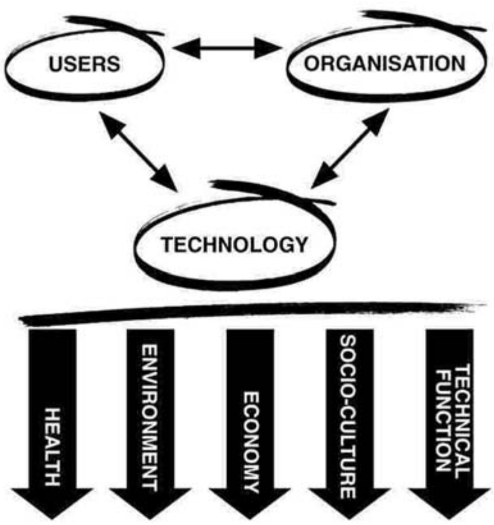

Figure 4. The Urban Water conceptual framework. At the top is the system with its sub-systems: technology, water users and organisation. The five sustainability aspects are represented by arrows (Urban Water, 2002).

The aim of the research in Urban Water was to develop support for strategic decisions on future water and wastewater systems in Sweden (Urban Water, 2002). This thesis is the result from one of the technical projects within the Urban Water program, where the aim was to evaluate the potential for integrated hydroponic systems as an alternative treatment method for domestic wastewater in Sweden. The discussion part in this thesis includes the sustainability criteria considering health, environment and technical function. A selection of associated indicators for these criteria, as presented by Hellström et al. (2000), is presented in Table 1.

Table 1. A selection of sustainability criteria with subcriteria and associated quantifiable indicators. Extracted from Hellström et al. (2000).

Criterion	**Indicator**
Health	
Risk of infection	number of waterborne outbreaks
Working environment	number of accidents
Environment	
Eutrophication	nitrogen to water
	phosphorus to water
Use of natural resources	area use
	energy source
	use of chemicals
	potential recycling of phosphorus
Spreading of toxic compounds to soil	recycling of excess sludge
Technical function	
Robustness	
Flexibility	

OBJECTIVES

The integration of hydroponic cultivation into a wastewater treatment system has been proposed as an ecological alternative to conventional wastewater treatment (Guterstam and Todd, 1990). In the hydroponic step, green plants can be cultivated that will increase the value of the system by means of valuable products (Todd and Josephson, 1996). The transformation of wastewater nutrients into products also provides an additional treatment pathway. Cultivation of plants in a temperate climate, such as in Sweden, implies that additional energy is needed during the colder and darker period. In conclusion, treatment capacity, additional energy usage and potential value of products are important aspects considering the applicability of hydroponic wastewater treatment systems in Sweden.

To enable the investigation of hydroponic wastewater treatment, a pilot plant was constructed in a greenhouse at Överjärva gård, Solna, Sweden. The pilot plant received and treated domestic wastewater from the Överjärva gård area. The primary goals of the Överjärva project were i) to achieve tertiary treatment effluent standards (15 mg BOD/L, 20 mg N/L and 0.5 mg P/L) without the use of chemicals, and ii) to recycle nutrients through extraction of useful products. The overall objective of the project was to evaluate the potential for integrated hydroponic systems as an alternative treatment method for domestic wastewater in Sweden. The project has been divided into three parts, and the main focus for the research presented in this thesis was the treatment aspect of hydroponic systems, mainly concerning organic matter and nitrogen removal.

The following questions laid the foundation for the studies and the discussion presented in this thesis:

- Which parameters are important for a successful outcome?
- How much energy must be used and how cost-effective is the process?
- How effective is the recycling of nutrients into valuable products?
- Is sludge production reduced?
- Which organisms work in the reactors?
- Is transparency significant for a treatment system?

The significance of transparency for a treatment system is included in the evaluation discussion. However, no research has been performed specifically in that area.

The experimental unit

The pilot plant was constructed with the purpose of treating the inflow domestic wastewater without the use of chemicals, and with recycling of nutrients through plant uptake. Conventional microbiological processes were thus combined with hydroponic cultivation. The system was composed of several parts (Figure 5); anoxic tank, aerated tank, three hydroponics (HP1-HP3), clarifier (CL), peristaltic pump (Pump 1), algal tanks, algal clarifier (ACL), sand filters and effluent pump (Pump 2).

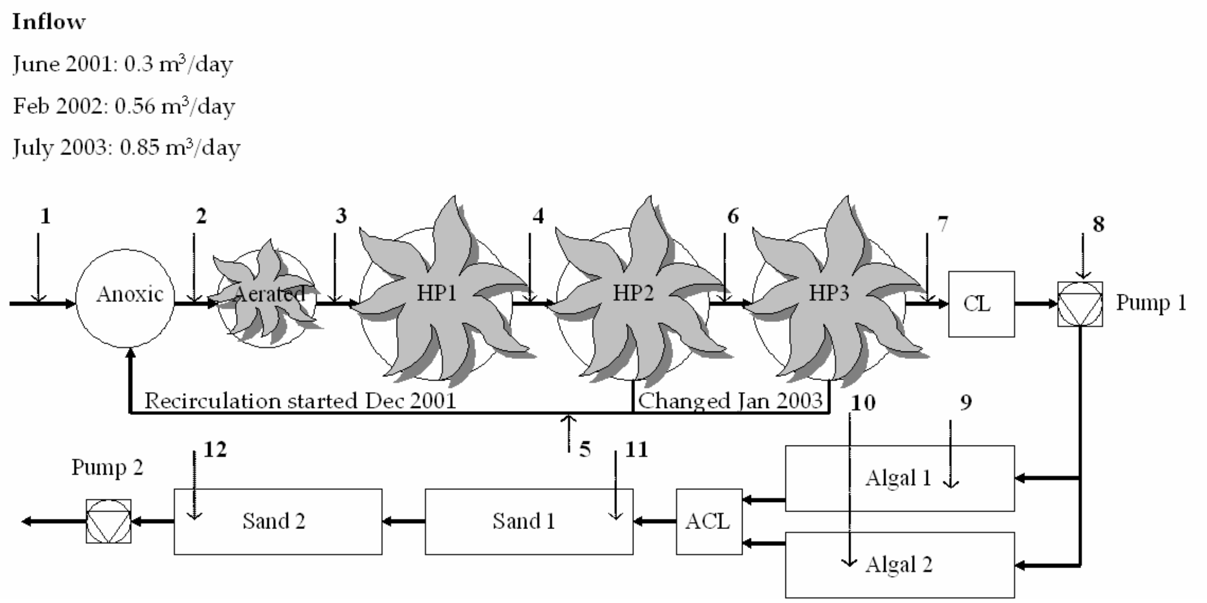

Figure 5. Overview of the tanks and flows involved in the system, including sample points 1-12.

The system was operated with pre-denitrification, thus nitrified wastewater was recirculated to the anoxic tank where the inflow carbon source was used for the denitrification process. The recycle flow was at first pumped from the second hydroponic tank, although later, in January 2003, the pump was moved to tank HP3. The anoxic tank contained 300 L of Kaldnes carriers (AnoxKaldnes, Norway), and the air-filled space was connected with the aerated tank. A planted soil filter was used as a cover on the aerated tank, and this solution has successfully prevented any wastewater related odor in the greenhouse. The hydroponics were intermittently aerated and used for cultivation of aquatic and terrestrial plants. The terrestrial plants were supported by a plastic net floating on the water surface in the tanks. The purpose of the clarifier was to sediment solids for subsequent recycling to the anoxic tank; however, solids were not accumulated in the clarifier, and hence, no recycling has taken place from this tank. The water flowed by gravity to the sump of the peristaltic pump, which was used to obtain continuous flow in the algal tanks. These tanks were constantly aerated to keep the algae in suspension and to provide CO_2 for algal growth. The sand filter tanks were 50% filled with sand and planted with vascular plants. Area and water volume for the respective tanks are presented in Table 2. Sampling has mainly been performed at the same points throughout the years (Figure 5). In average, sampling and analysis were performed twice per week, except during the intensive two studies, July 2002 and February 2003, when daily samples were analysed. Sampling time has been around 9.30 am, following a pumping cycle of inflow to the system. A more detailed description of the system and its components can be found in Paper I.

Table 2. Area and water volume of the respective tanks in the system.

	Area [m^2]	Water volume [m^3]
Anoxic tank	0.74	0.59
Aerated tank	0.74	0.59
HP1	1.96	1.57
HP2	1.96	1.57
HP3	1.96	1.57
Clarifier	0.33	0.14
Algal 1	1.62	0.50
Algal 2	1.62	0.50
Algal clarifier	0.67	0.20
Sand 1	1.11	0.09
Sand 2	1.11	0.09

Basic requirements

A fundamental difference between conventional wastewater treatment and hydroponic treatment is the introduction of green plants into the system, thus, the need for additional light and heat to a hydroponic system are correlated to the geographical position. Based on literature, two operational conditions were defined: "good growth" and "minimum requirements" (Paper III).

Good growth conditions were defined as sufficient temperature and light to maintain growth by plants with moderate light requirements: minimum 20°C, 2000 lux 16 h day^{-1}. The minimum requirements were sufficient to maintain winter-resting plants without growth: minimum 10°C, 400 lux 16 h day^{-1}. The two conditions were subsequently used to calculate the monthly theoretical energy requirements for hydroponic wastewater treatment in Stockholm as well as four other Swedish cities: Lund (55°72' N), Visby (57°38' N), Stockholm (59°35' N), Östersund (63°20' N) and Kiruna (67°83' N). For the calculations, values for day average temperature and hour mean global irradiance (W m^{-2}) aquired from SMHI (Swedish Meteorological and Hydrological Institute) were used. The results show that the total energy demand is dominated by energy for heating, and that the heat demand significantly increases with latitude (Figure 6).

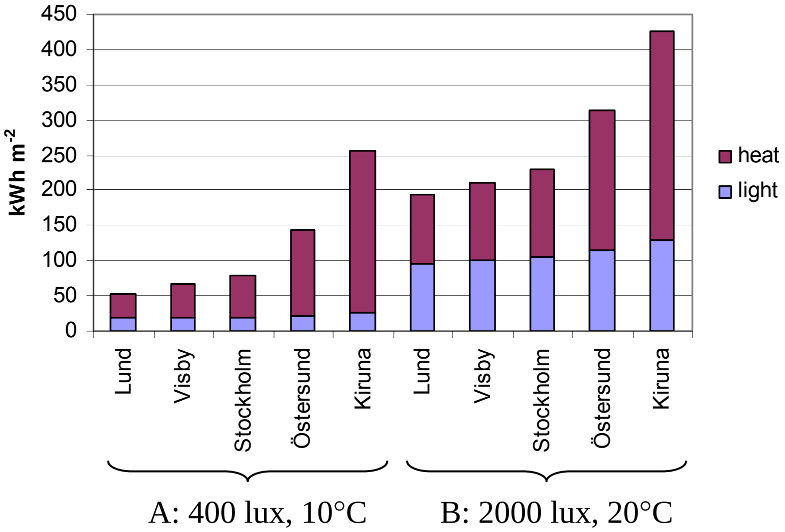

Figure 6. Theoretical total heat and light requirements in one year for five Swedish cities. A = "minimun requirements", B = "good growth" (Paper III).

Operation and results

Over the years, the flows have been modified several times. Studies included in Paper I and II were performed when the inflow was 0.56 m^3/day, and the recycle ratio was around 2 and pumped from the second hydroponic tank, HP2. During the winter of 2002/2003 the greenhouse environment was adjusted to resemble the conditions defined for minimum requirements for plant growth (Paper III). The aim was to evaluate the effects on the treatment capacity as well as the general condition in the greenhouse. In January the recycle ratio was increased to around 3 and pumped from tank HP3, and in February the second intensive study was performed. Samples for the FISH-study (Paper VI) were taken when the inflow had been increased to 0.85 m^3/day and the recycle ratio decreased to around 2 and then later to around 1.

In summary, the results from the July study (Paper I) showed that the system reached the design effluent targets of 90% COD removal, complete nitrification and removal of total nitrogen as expected with a recycle ratio of around 2, however, the typical standard effluent limits of around 10-15 mg BOD/L and 10-20 mg N/L (VAV, 2001), were not always reached with the present set-up. Phosphorus was removed by 47% and the majority was detained in the algal step and sand filters. Although with an inflow concentration of about 15 mg P/L the standard effluent limit of 0.2-0.5 mg P/L (VAV, 2001) was still out of reach. During the intentionally cooler winter period, the plant growth almost ceased as expected; but the analysis showed that the overall treatment capacity did not significantly change during this period.

Organic matter

Concentrations of organic matter was generally measured as chemical oxygen demand (COD). The biochemical oxygen demand (BOD) was also measured for the inflow during the study in July 2002 (Figure 7). In addition, occassional control measurements of BOD levels in the sump of Pump 1 were performed. The measured levels of COD in the effluents from the three hydroponic tanks and in the sump for Pump 1 were almost equal, which might indicate that the majority of biological degradable organic matter was already removed after the first hydroponic tank. The inflow amount of COD was 50% higher during the February study. The main difference was the removal in the aerated tank, which was almost twice as high compared to the July study, however, the effluent amount was about the same. In total, the system reduced 90% of COD in July and 85% of COD in February.

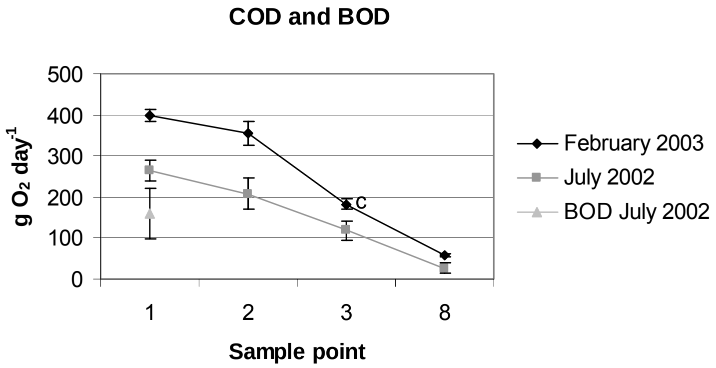

Figure 7. The amounts of COD and BOD in the inflow (1), and the decrease in amount of COD between the different sampling points: out from the anoxic tank (2), out from the aerated tank (3) and in the sump of Pump 1 (8). Error bars indicate standard deviation. February 2003 n=12; July 2002 n=22; BOD July 2002 n=5.

Nitrogen

The inflow concentrations of nitrogen to the system has been rather high, around 95 mg N/L. Compared to the reference values of raw wastewater (Henze et al., 2002), this indicated a concentrated wastewater. In the anoxic tank the inflow was mixed with recirculated nitrified wastewater. The effluent from the tank, sample point 2, had low levels of nitrate, thus the denitrification process was working (Figure 8). Almost no nitrification was detected in the aerated tank, instead nitrification occurred in the hydroponic tanks. During the July study, complete nitrification, effluent levels less than 1 mg NH_4-N/L, was achieved in tank HP2 (Paper I). After the recycle ratio was increased in January 2003, the levels of ammonium in the effluent from tank HP1 increased with a subsequent increase in tank HP2; therefore the recycle pump was moved from tank HP2 to HP3. With respect to mass-flows, the same amount of ammonium was nitrified in tank HP1 during July, and tanks HP1 and HP2 during February, around 27.5 g NH_4-N per day. All ammonium was converted to nitrate and no nitrite accumulation was observed.

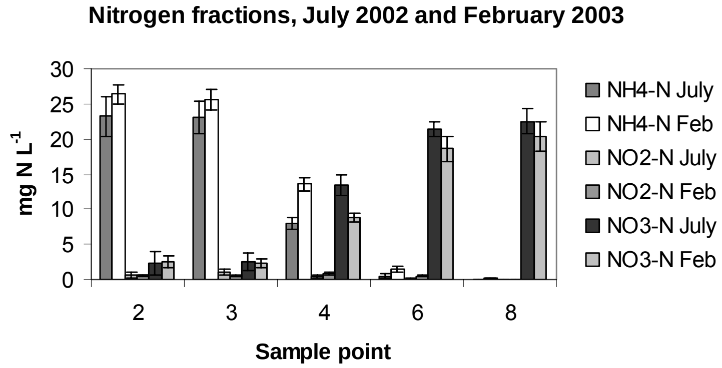

Figure 8. Nitrogen fractions in the effluents from the anoxic tank (2), the aerated tank (3), HP1 (4), HP2 (6) and in the sump of Pump 1 (8). Concentrations are presented from the July and February studies, the average water temperature was 22°C and 11°C respectively. Error bars indicate standard deviation. July 2002 n=10; February 2003 n=12.

Phosphorus

The concentration of total phosphorus was measured throughout the whole system during the July study. The inflow concentration was on average 15 mg P/L. Around 4.5% of the total amount of phosphorus passing the anoxic tank was captured in the tank. The removal through uptake by plants and assimilation by bacteria in the hydroponics was around the same amount. The most effective reduction was achieved in the parallel algal tanks, which in the first set-up removed 27.6% of the incoming amount of phosphorus to the step. This removal was proposed to be due to a combination of algae assimilation and chemical precipitation induced by the high pH in the algal tanks (Figure 9). In total, 47% of the amount of inflow phosphorus was removed in the system (Paper I).

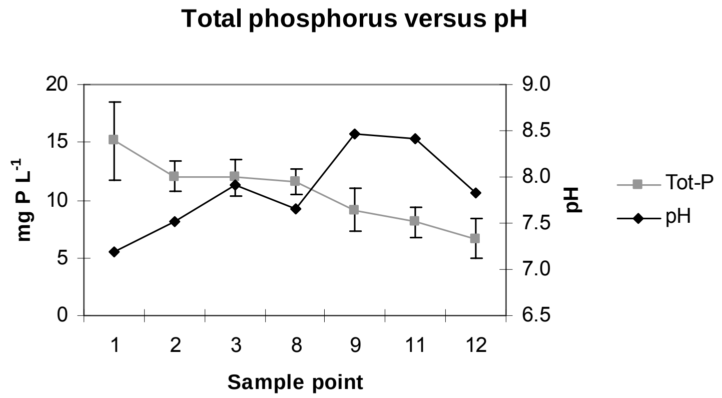

Figure 9. Total phosphorus and pH at different sample points. Inflow (1), effluent from the anoxic tank (2), the aerated tank (3), the sump of Pump 1 (8), Algal 1 (9), start of Sand 1 (11) and end of Sand 2 (12). Error bars indicate standard deviation, n=10 (Paper I).

In the February study, the average inflow concentration was around 12 mg P/L. The decrease in concentration between the inflow and in the sump of Pump 1 was around 10%, compared to a 23% decrease in the July study. During the summer of 2003 the sand filter tanks were converted to algal tanks. Since then, the main removal pathway for phosphorus in the system is part of the microalgae research, which will be presented in the coming doctoral thesis by Karin Larsdotter.

Comparison with systems found during the literature review

A literature review has been performed to identify existing similar wastewater treatment systems, i.e. systems including hydroponics and/or aquacultures located in a greenhouse environment. In total, eight systems were found, of which five had published enough data to enable a comparative study (Paper IV). In general, the main components were similar, although the algal step was specific for the Överjärva system. The six systems, including Överjärva, can be divided into three subgroups: i) demonstration systems with small inflow and long hydraulic retention time (HRT) [1, 5], ii) pilot systems with small to moderate inflow and moderate HRT [2, 3, 6], iii) full scale system with large inflow and short HRT [4] (Table 3).

Table 3. Inflow and hydraulic retention time for the studied systems.

	inflow [m³/day]	**HRT [days]**
[1] Nordic Folkecenter[A]	3-4	30[B]
[2] Harwich – Solar Aquatic Systems© [C]	9-10*	7.5
[3] Providence – Living Machines® [D]	34	4.5
[4] South Burlington – Living Technologies, Inc.[E]	300	2.8
[5] Stensund wastewater aquaculture[F]	5-6[F,G]	24.5
[6] Överjärva[H]	0.6	12.7

* inflow is an approximation based on specified volumes and HRT for the Harwich system. A) Hinge and Hamish, 1997; B) Boisen, 1995; C) Peterson and Teal, 1996; D) Todd and Josephson, 1996; E) Austin, 2000; F) Guterstam, 1996; G) Guterstam et al., 1998; H) Paper I.

Due to the large variations regarding inflow and HRT, the removal of organic matter, nitrogen and phosphorus was compared, based on the difference between influent and effluent concentrations, rather than removed amounts per day (Figure 10). The conclusions were that the three systems with the shortest HRTs [2, 3, 4] showed the highest daily removal of BOD. Considering that the depth of their aerated tanks were 1.4 m or more, and that [2] reportedly contained solids in suspension, the biological process responsible for removal of BOD in these systems probably resembled an activated sludge process. The three systems with the highest removal of nitrogen had internal recirculation [4, 6] or added an external carbon source to the process [2]. Most of the removed phosphorus was due to sedimentation with subsequent removal, and adsorption in the system. None of the systems reached the Swedish standard effluent limit of 0.5 mg P/L.

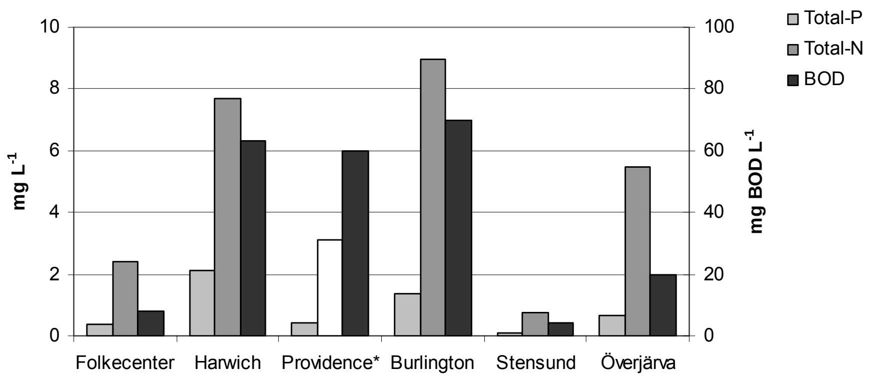

Figure 10. Removal per day in the different treatment plants. BOD values on the right y-axis. * Nitrogen presented as ammonia concentrations (Paper IV).

Operation alternatives

During the February study, the activity of the green plants was low; however, the treatment capacity of the system remained. The conclusion is that active plant growth is not necessarily required for a successful treatment performance. For the Överjärva system, the theoretical energy demand to keep the 60 m^2 greenhouse environment at good growth conditions all year around would be about 9000 kWh more than to satisfy the minimum requirements. This in the same range as the amount of energy needed for treatment, i.e. 8760 kWh $year^{-1}$ for supplied aeration (Paper V). If treatment is the main purpose, then the strategy of keeping the plant activity low during the cold and dark season would be an alternative to decreasing the energy usage. If the system is used for growing a defined product, however, then a prolongation of the growth period might be beneficial, and a couple of extra productive months per year can be gained without much extra energy use.

Pathogen removal

In domestic wastewater, there are several infectious organisms that may cause diseases in humans, e.g. bacteria, protozoa and viruses (Bitton, 1999). The pilot plant did not include a disinfection step, thus the removal of pathogens in the system was an interesting subject. In October 2002, a study to measure the microbial removal of the system was performed in cooperation with Jakob Ottoson at SMI (Smittskyddsinstitutet, Swedish Institute for Infectious Disease Control).

Traditional indicator bacteria for faecal contamination are coliforms, *Escherichia coli* and enterococci. However, several studies have shown that the removal of protozoa and viruses differ from that of bacterial indicators (e.g. Bonadonna et al., 2002). Other indicators are therefore necessary to determine the removal of these organisms. Previous studies have successfully used *Clostridium perfringens* spores as an indicator for (oo)cyst removal (Payment and Franco, 1993; Hijnen et al., 2000). Somatic coliphages have been proposed as an indicator to determine the removal of human enteric virus (Havelaar et al., 1991). Five indicators were thus analysed to determine the removal of microorganisms in the Överjärva pilot plant.

In a hydroponic treatment system, there are several aspects to pathogen exposure, such as: what are the risks associated with i) working with the system, ii) discharging the effluent, and iii) using the products from the system. In this study, the two first questions were addressed, and samples were taken from four points: (In) the inflow to the pilot plant, (1) the influent to the first hydroponic tank, i.e. the first open tank in the system, (2) in the sump of Pump 1, and (Out) the effluent from the pilot plant (Figure 11).

In general, removal in the first and last part was higher than in the hydroponic step. The removal in the first two tanks was probably due to the anoxic conditions and/or the higher biomass in these tanks. In addition, there were some dilution effects due to the recirculation flow. Concerning spores and coliphages, however, the results showed only slight removal in that part, between sample point In and 1 (Figure 11). Safety measures should therefore be considered regarding human contact with wastewater in the early open part of the system.

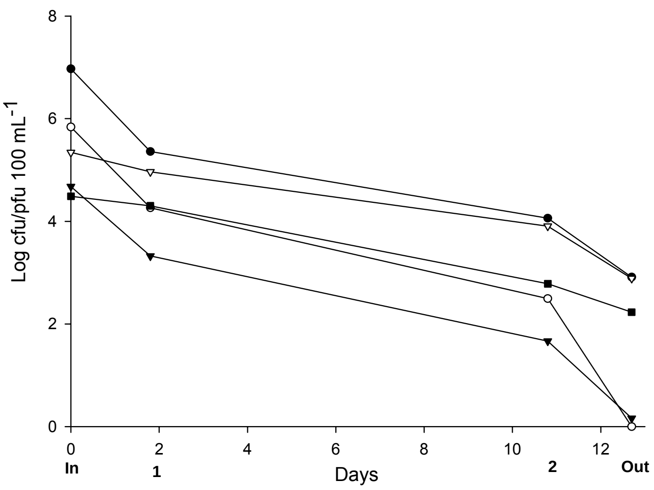

Figure 11. Log number of faecal indicators in hydroponics wastewater treatment as a function of treatment and hydraulic retention time. Total coliforms (●), *E. coli* (○), enterococci (▼), somatic coliphages (▽) and spores of sulphite-reducing anaerobes (■) were measured after the anoxic and aerated tanks (1), hydroponic treatment (2), algal tanks and sand filters (out) (Paper II).

A major part of the overall removal occurred in the last part, where adsorption to the sand filters and elevated pH in the algal tanks were considered to be contributing factors. *E. coli* was never detected in the effluent after more than 5.8 log removal. This figure can be compared to the removal of 2-3 log for the same parameter in common Swedish wastewater treatment plants for secondary treatment (Stenström, 1986). Enterococci was detected in 1/3 of the effluent samples after more than 3.8 log (99.98%) reduction, and coliforms showed a 4.1 log reduction. Spores and coliphages were not as efficiently removed as the bacterial indicators. Spores followed a time-dependent loglinear removal decay rate. Due to the high HRT (12.7 days) a 2.3 log removal was achieved, compared to 1.3 log in secondary treatment (Stenström, 1986). Spores are not likely to settle as easily as (oo)cysts and could therefore be regarded as a conservative process indicator for (oo)cyst removal. Coliphage removal was also higher than secondary treatment, 2.5 log and 1.3 log respectively (Stenström, 1986). The mean decay rate in the first and hydroponic part was similar to what Gantzer et al. (2001) reported in standing wastewater at 22°C. In conclusion, all the analysed indicators were removed satisfactorily and the achieved effluent quality was comparable to, or better than, for conventional secondary treatment.

System microbiology

The tanks in the pilot plant provide different environments for microbial life. The level of dissolved oxygen varies as well as the concentrations of nutrients and organic matter. The hydroponic step was considered to be the most interesting part of the process, hence the emphasis on identification of the microbial composition there. The first hydroponic tank (HP1) was chosen as the sample point, since it had the highest influent concentrations. The general microbial composition was investigated during a six-months period (December 2003 to June 2004) by means of fluorescent *in situ* hybridization (FISH, Appendix B), using a set of 32 different general, as well as specific, probes. During the sampling period, general conditions in the tank were: pH 7.5-7.9, oxygen level 1.9-3.9 mg O_2/L, water temperature 15-25°C and total phosphorus around 10 mg P/L. Levels of nitrogen and organic matter in the domestic inflow and outflow of tank HP1 are presented in Table 4.

Table 4. Levels of nitrogen and organic matter in the domestic inflow and outflow of tank HP1 during the period December 2003 to June 2004. For nitrogen fraction measurements n=41. For Tot-N and COD n=19 (Paper VI).

	Tot-N [mg N/L]	NH_4-N [mg N/L]	NO_2-N [mg N/L]	NO_3-N [mg N/L]	COD [mg O_2/L]
in	72 ± 13.0	36 ± 10.9	1.9 ± 0.85	13 ± 7.0	132 ± 20.5
out	54 ± 6.7	19 ± 7.6	1.4 ± 0.82	34 ± 8.6	111 ± 16.0

Representatives of the two prokaryotic domains could be found. Cells within *Bacteria* dominated clearly over cells within *Archaea* in all investigated samples (Table 5). Attempts were also made to identify cells within the *Eukarya* domain but yielding mostly unspecific signals or weak fluorescent signals in larger cells. The fixation protocols used for prokaryotic cells is not optimal for eukaryotic cells, e.g. ciliates, flagellates and entamoebae. For a more in depth study of the *Eukarya* domain, other fixation protocol should be used. It is nevertheless obvious that prokaryotic cells dominated over eukaryotic cells. Further, the most abundant phyla were the *Proteobacteria* and the *Planctomycetes*.

In the hydroponic step, nitrification had previously been identified as the dominating process. Ammonia oxidizers belong to the beta-subclass of *Proteobacteria*, which also was identified as the most abundant proteobacterial subgroup (Figure 12). The main ammonia oxidizers in tank HP1 were identified as the *Nitrosomonas oligotropha*-lineage, while the nitrite oxidizers belonged to the *Nitrospira* spp. Traditionally, *Nitrobacter* was considered to be the most important nitrite-oxidizer in wastewater treatment. Today, the importance of *Nitrospira*-like nitrite oxidizing bacteria is well confirmed (e.g. Daims et al., 2001), and the dominance of the different species in different environments are considered to be a reflection of their different survival strategies (Wagner et al., 2002). The adaptation of *Nitrospira* spp. to low oxygen and nitrite concentration has been shown by Wagner et al., 2002. Since the water in tank HP1 had low nitrite levels (Table 4), the finding of *Nitrospira* spp. as the prevalent nitrite-oxidizer is well supported by current knowledge. The target organisms for the individual probes included in Table 5 can be found in Paper VI, together with optimal formamide concentrations, sequences and target sites.

Table 5. Occurrence of cells identified with different oligonucleotide probes during an investigation period of six months (December 2003 to June 2004). Results are presented according to a five-graded scale, since no quantitative analyses of the probe-defined microbial groups could be made due to weak back-ground autofluorescence: always abundant (+ + + +), abundant when present (+ + +), present but in less amounts (+ +), sporadic (+) and not detected (-). The regularity of occurrence throughout the period of six months is also included (Paper VI).

	Probe	Occurrence	Regularity
Domain: *Archaea*	ARCH344	+ +	constant
	ARCH915	+ +	constant
	CREN499	-	-
	EURY498	+ +	constant
Domain: *Bacteria*	EUB338	+ + + +	constant
	EUB338 II	+ + + +	constant
	EUB338 III	+ +	constant
- Proteobacteria	ALF968	+	sporadic
	BET42a	+ + +	significant change[a]
	BONE23A	+ + +	constant
	BTWO23A	+	constant
	GAM42a	+ +	constant
- Nitrifying Bacteria	NIT3	-	-
	Nso1225	+ + +	significant change[a]
	Nmo218	+ + +	significant change[a]
	NmV	-	-
	Ntspa712	+ + +	constant
	Ntspa662	-	-
- Gram-positive Bacteria	HGC69A	+ +	significant change[a]
	LGC354mix	+ +	significant change[a]
- Other taxa	PLA46	+ + + +	constant
	PLA886	+ + + +	constant
	CF319a	+	sporadic
	CFX1223	-	-

a) most likely due to the accidental washout, reoccurrence after two weeks.

During the course of the study, in January 2004, the pilot plant experienced a "washout". In one of the houses within the Överjärva catchment area a fresh water tap had accidently been left open, resulting in significant dilution of the inflow wastewater. As a result of the decreased ammonium concentration in the inflow, decreased nitrification activity was measured in the system. Further, analysed FISH samples showed lower probe signal intensities, especially concerning positive clusters in samples hybridized with probes targeting *Betaproteobacteria* and betaproteobacterial ammonia-oxidizing bacteria. After the concentrations had returned to previous levels in tank HP1, however, the nitrification activity seemed to recover within a two week period.

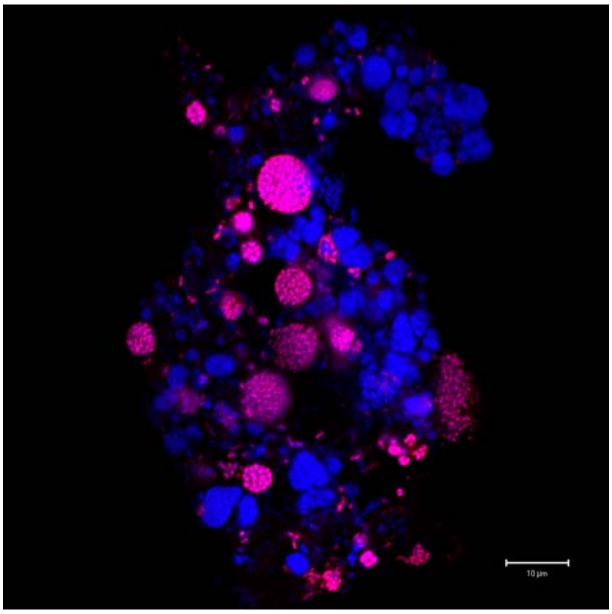

Figure 12. Overlapping FISH image in sample from December 2003, based on two probes: EUB338 mix (Cy5, blue) and BET42a (Cy3, red). The overlapping probes make EUB cells targeted by Bet42a pink. The inserted scale bar represents 10 µm.

DISCUSSION CONCERNING THE RESULTS

Processes in the separate steps

The anoxic tank

The Överjärva pilot plant was operated as a pre-denitrification system, i.e. the first tank was anoxic, and organic matter in the inflow was used as the carbon source for the denitrification process. In denitrification, heterotrophic bacteria uses nitrate instead of oxygen as the final electron donor in the respiration process; thus, nitrate is converted into free nitrogen while organic matter is oxidized, i.e. degraded. Denitrification is dependent on several environmental factors such as energy source, temperature, oxygen levels and pH (Henze et al., 2002). When inflow organic matter is used, the hydrolysis process will often be the rate limiting step. During hydrolysis, larger molecules are converted into smaller, directly degradable molecules. The proportional relation between the carbon source and nitrogen, the C/N ratio, in the influent into the tank is also important for a successful process, since inadequate amounts of organic matter will limit the denitrification process. The influence of excessive oxygen was experienced during a period when the recycle flow had high levels, around 6-8 mg O_2/L, which temporarily inhibited the denitrification process.

During the study in July 2002, denitrification was working as expected, with an inflow of 0.56 m^3 per day and a recycle ratio of 2.26 (Paper I). It was also concluded that the denitrification process was limited by the nitrate concentration rather than the organic matter, i.e. the nitrogen reduction was limited by the recirculation rate. Microbiological processes are temperature dependent and can be described in an exponential expression for the range 0-32°C (Henze et al., 2002). So, for every 10°C increase, the process rate is doubled. During the study in February 2003, the recycle ratio had been increased to 2.97 and denitrification was still achieved as expected. Although it should be noted, that during a few days when the temperature was below 10°C in the water, the nitrate concentration in the effluent from the anoxic tank was somewhat higher than normal.

The C/N ratio between removed organic matter and removed nitrogen in the anoxic tank was around 2, which is considered low. This might indicate that additional carbon was provided through degradation processes of accumulated biomass in the tank. This is a probable explanation due to the fact that no biomass was removed from the tank, and that some oxidation must take place with the dissolved oxygen provided with the recycle flow. This is also consistent with the measured levels of ammonium in the influents and effluent, which when calculated into mass-flows showed that the influent amount of ammonium to the tank was less than the effluent amount.

The aerated tank

The main purpose of the second tank was to aerate the effluent from the anoxic tank. In addition, the lid of the tank was a constructed compost filter which successfully prevented any odor in the greenhouse. The results indicated that the supplied oxygen was used solely by heterotrophic bacteria, since no nitrification was observed in this tank (Paper I). The level of dissolved oxygen has been around 4.5-6.5 mg O_2/L, and the majority of the total COD removal took place in this tank. The removed amount of COD, however, was twice as high during the February study compared to the July study. This was most likely due to the higher amount of inflow COD to the system during February. One explanation for the increased inflow COD might be the cold outdoor temperatures, which probably effected the processes in the equalising tank more than the relatively small temperature change for the processes located indoor. In total, 34% and 43% of the total removal occurred in the aerated tank during July and February respectively. Aeration was supplied through a single airstone, which probably significantly limited the conversion yield. In conclusion, improved aeration, possibly in combination with a longer retention time in the tank, would increase the overall removal of organic matter in the system.

The hydroponics

Through the results from the water analysis, it was obvious that nitrification occurred in the hydroponic step. Since there was almost no biomass on the bottom of the tank, the search for activity started at the roots. The very first investigation included a 300 ml sample from tank HP1, which included 95.0 cm of *Canna* sp. roots with attached biofilm. Roots and biofilm were carefully separated by washing. Both samples were given a start concentration of 22 mg NH4-N/L and the ammonium oxidation was followed photometrically at 19°C. Good removal occurred in the sample with biofilm (Figure 8) whereas the root-sample showed only minor removal. In the biofilm sample, the dry matter content was high, 10 mg SS/ml, and the calculated nitrification rate was found to be 1.12 mg NH_4-N (g SS*h)$^{-1}$. Later studies have measured the nitrification rate to be around 1.4-1.6 mg NH_4-N (g SS*h)$^{-1}$ which correspond well to the first investigation (Paper VI).

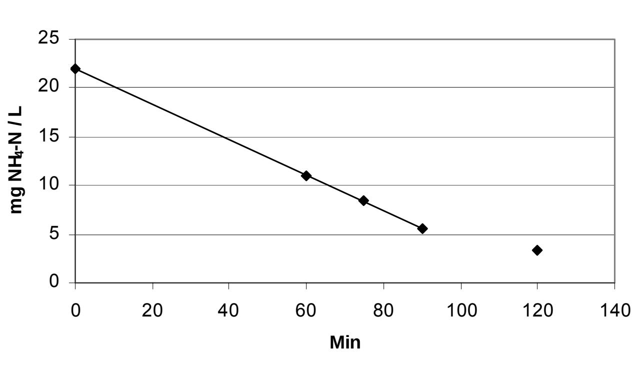

Figure 13. Ammonium removal in the biomass sample, January 2002.

As for the denitrification process, the C/N ratio is important for nitrification. A high ratio will favor the growth of fast growing heterotrophs, which in turn will prevent the growth of the slower autotrophic nitrifyers. In the hydroponic tanks HP1-HP3 the measured COD levels were almost constant, and the calculated COD/NH_4-N ratio in the tanks were approximately 3.5-4, 5-6 and 25 respectively. Nitrification was mainly achieved in tanks HP1 and HP2 where the ammonium concentrations were high. Compared to the nitrification rates for an activated sludge process, the measured rates are rather low. For example, for a C/N ratio (g BOD_7/g Tot-N) of 3.5, the expected nitrification rate is 2 mg NO_3-N (g VSS*h)$^{-1}$ in an activated sludge reactor at 10°C (Ødegaard, 1992).

The two main design purposes of the hydroponic step were: i) to provide an aerated environment for the conversion of organic matter and the nitrification processes, and ii) uptake of nutrients through growth of macrophytes. Other similar, but full-scale systems, have been operated as nitrifying activated sludge systems (Todd et al., 2003), whereas in the Överjärva system, there was no significant accumulation of suspended solids in the hydroponics. Instead the biofilm associated to the plant roots was shown to be the location of the nitrification activity, which implies that the amount of roots in a tank is highly significant for the process rate. As a result, the Överjärva system was considered comparable to a biofilm system, rather then a nitrifying activated sludge system regarding process kinetics.

The hydroponic tanks were aerated to provide the plant roots with oxygen and to promote nitrification. Excessive oxygen levels in the recycle flow, however, proved to be inhibiting on the denitrification process during high recycle ratios. Aeration in the three hydroponic tanks was therefore independently controlled to supply enough oxygen for nitrification in tanks HP1 and HP2, and to provide air mainly for mixing purposes in tank HP3. Considering the multiple processes that utilized the supplied oxygen, i.e. nitrification, heterotrophic conversion and root respiration, it is very likely that the actual dissolved oxygen levels in the tanks were in fact limiting to the nitrification process. The oxygen levels in tanks HP1 and HP2 were around, or less than, 4 mg O_2/L, which is far from optimal for the nitrification process in a biofilm (Ødegaard, 1992). Considering that the plant roots occupied approximately 25% of the tank volume and that about 27.5 g NH_4-N was nitrified per day, the nitrification rate can also be expressed as around 0.07 kg N per day and m^3 active reactor volume. This rate is below the range of rates, 0.2-2.0 kg N/m^3/d, reported for other nitrifying biofilm systems (e.g. Rusten, 2000; Bonomo, 2000). In conclusion, the system was not operated as a high rate nitrifying process. Although, with the current set-up the target, complete nitrification, was reached.

Another important aspect of the hydroponics is the long retention time in this step. Out of the system's total retention time of 12.7 days, 67% of the time, or 8.4 days were spent in the hydroponic tanks. The main purposes of the tanks were the microbial processes and nutrient uptake through plant growth. Hence, active reactor volume could be considered as the approximately 25% occupied by plant roots. It is likely to assume therefore that the same nitrification rate could be reached with shallower tanks. For a system aiming at producing green biomass, arable area is more important than tank volume. Thus, several shallower tanks would imply more arable area, and would still provide the system with adequate retention time to achieve complete nitrification. In addition, in a system with a large accessible area, products from the system might contribute more to the treatment through uptake removal. In the long run, this is of course a matter of available space and economic value of the produced biomass.

The algal tanks and planted sand filters

In the preceding steps, organic matter and nitrogen were efficiently removed, however, the expected removal of phosphorus was low. Results showed that during the July study, around 9% of the total amount of phosphorus was removed, whereas during the February study, the removal was even less. The main design purpose of the algal tanks and the planted sand filters was to remove phosphorus from the effluent. Although the removal yield in the sand filters was higher than the anoxic tank and hydroponics, the removed amount in these three steps was comparable due to the lesser daily water volume, i.e. amount of phosphorus, passing the sand filter. The most effective removal was achieved in the algal tanks. In conclusion, removal through plant uptake of phosphorus in a small hydroponic system is insignificant. Adequate adsorption steps or other biological alternatives are required to achieve effective phosphorus removal in a system without chemicals.

Recycling of nutrients

Nitrogen and phosphorus are essential macro-nutrients for plant growth. Nitrogen is an important element in cellular proteins, and phosphorus has a key position in cell energy transfer. Most often they are assimilated as simple inorganic ions (Forsberg, 1991). Nitrogen, in the form of nitrate or ammonia, is readily assimilated by higher plants. Ammonia or ammonium ions are rapidly incorporated into organic compounds, whereas nitrate must be reduced to ammonia prior to assimilation. Ammonia, however, is not accumulated in the plant since high levels are toxic to most plants. The main assimilation path-way is through production of glutamate, which then is further incorporated into other amino acids. Phosphorus is taken up by plant roots as phosphate, and the main entry point occurs during the formation of ATP. Once incorporated in ATP, the phosphate group can be transferred in reactions to form the various phosphorylated compounds found in higher plants (Taiz and Zeiger, 1991). In very productive plants, such as wetland plants, considerable amounts of nutrients can be bound in the biomass. The uptake capacity of emergent macrophytes is approximately 20 times higher for nitrogen than for phosphorus (Brix, 1994). This reported ratio is much higher than the traditional Redfield ratio for algae, which is 7:1, based on weight (Redfield, 1958).

During the first study in July 2002, the calculated removal of phosphorus in the hydroponic step was small, and credited to assimilation by plants and the microbial biofilm (Paper I). During the following sampling periods, no significant reduction of phosphorus was recorded in the hydroponic step. In addition, the partition between plant and bacterial assimilation in the system has not been investigated. The uptake by plants as well as by microorganisms therefore, can not be quantified without a controlled study of biomass production in the system. Considering the removal of nitrogen, the achieved additional removal, compared to the theoretical amount removed by the defined recycle ratio, was credited to plant uptake (Paper I). The amount was approximately 4% of the nitrogen inflow, which was comparable to results presented by Peterson and Teal (1996). The actual uptake, however, was not measured.

The system has not been operated as a production system and the vegetation was randomly harvested, mainly to prevent the plants from becoming too crowded or tall. During periods of substantial harvesting, the amounts removed have been recorded per species. Since there has been no consistent recording of added plants or biomass removed during occasional harvesting, it is not possible to elaborate on the plant specific uptake within the scope of this thesis. Although it is possible to conclude that during the first four years of operation, there has been no significant recycling of nutrients by means of transformation into valuable products in the hydroponic step.

The hypothetical potential, however, using tomatoes as the cultivated product can be calculated. Jungersen (1997) used wastewater from eel production to cultivate tomatoes in a nutrient film system. In the nutrient film technique (NFT), a very shallow film of solution is used, and the solution is recirculated to provide a constant flow over the roots of the plants. The used wastewater solution had nutrient concentrations similar to the Överjärva inflow, 100 mg N/L and 10-15 mg P/L. The annual rates of nitrogen and phosphorus removal for tomato plants were 338-429 mg N/m^2/d and 52-70 mg P/m^2/d. The annual yield of tomatoes was around 30 kg/m^2 (Jungersen, 1997). Suppose that the hydroponic tanks, total area around 6 m^2, were used for cultivating tomatoes, with the following prerequisites: i) hydroponics and NFT cultivation are comparable, ii) the removal rates are comparable although the nutrient concentrations are lower in the hydroponics, and iii) the tomato plant roots are adequate for the nitrifying biofilm. The annual yield from the system would then be around 180 kg tomatoes, and the removed amount of nitrogen and phosphorus would be approximately 4-5% of the inflow amounts. If tomato cultivation through NFT was the only treatment available, then 120-160 m^2 would be necessary for removal of the daily nutrient amounts in 0.56 m^3 of the Överjärva inflow.

EVALUATION OF THE ÖVERJÄRVA SYSTEM

How can we judge if a treatment system is sustainable? Sustainability is a complex issue, which is impossible to measure. As a result, discussions concerning sustainability often deal with different criteria where some are quantifiable, such as water quality issues, while others can only be relatively compared between different applications (Hellström et al., 2000). Wastewater treatment in systems combining microbiological processes with hydroponic cultivation, and in some cases aquacultures, are claimed to use solar energy as their primary energy source (Todd, 1997). These systems are claimed to provide little or no sludge, generate useful byproducts, use no hazardous chemicals in the process chain and remove synthetic chemicals from the wastewater (Guterstam and Todd, 1990). These benefits will be further elaborated on here based on results and experiences gained from the Överjärva pilot plant and the papers included in this thesis. Removal of synthetic chemicals will not be discussed, since they have not been part of the experimental protocol. The discussion will start with quantifiable criteria, which in the field of wastewater treatment might typically include environment and health issues.

Traditionally wastewater treatment has primarily concerned observed pollution effects such as oxygen depletion and eutrophication in receiving waters. As a result, standardized effluent concentrations have been set and thus a successful treatment system can be partly defined by its effluent characteristics. On the other hand, the required effluent standard may vary depending on local conditions and type of receiving waters. Today, there are systems that might suffice in some areas, while in others they will not. Included in the environmental quality objectives adopted by the Swedish parliament in 1999, is the zero emission objective (Miljömålen, 2004). Although this is a long term goal, it would be desirable that all treatment systems aimed for at least the effluent standards of 10-15 mg BOD/L, 10-20 mg N/L and 0.2-0.5 mg P/L, as defined in VAV (2001). Besides treatment performance in relation to effluent standards, several other measurable parameters are included in the environment criteria, such as:

- energy consumption and type of energy source
- waste production, e.g. sludge
- use of chemicals
- area use

There are many parameters that can be measured and discussed in relation to wastewater, however, treatment performance in this discussion is limited to the concentrations of organic matter, nitrogen and phosphorus in the effluent.

A hydroponic wastewater treatment system includes many of the steps found in conventional treatment plants as well as hydroponic cultivation. Typical steps are primary treatment, anoxic parts, aerated parts, clarifiers/sedimentation and final polishing. The achieved degree of treatment is therefore mainly dependent on the construction of the system and the same conditions that apply for other biological systems. Thus, removal of organic matter is rather straight forward as long as adequate amounts of bacteria are present and sufficient aeration is provided. Depending on the construction, nitrification in a hydroponic system can be achieved in an activated sludge manner (Todd, 2003), or in a plant root associated biofilm (Paper VI). The two systems perform equally well, although a biofilm system requires higher oxygen levels in the water body due to biofilm kinetics (Henze et al., 2002). The denitrification process also follows the same conditions as for conventional large scale treatment. Pre-denitrification where organic matter in the inflow is used, has proven to work well (Austin, 2000; Paper I). Post-denitrification requires addition of an additional carbon source (Henze et al., 2002). In the Harwich septage treatment pilot facility (Paper IV), calibrated amounts of acetate were regularly added to achieve denitrification without increasing BOD in the effluent (Peterson and Teal, 1996); however, from a sustainability view-point, supplementary carbon sources should be avoided.

The general trend for wastewater treatment is to use less chemicals through transition to biological methods (VAV, 2001). Removal of phosphorus without chemicals, however, is a tricky question, especially regarding the high concentrations of phosphorus that were measured in the inflow at Överjärva. On average, the inflow had a concentration of 15.1 mg Tot-P/L (Paper I), compared to the average inflow of 4.6-7.4 mg Tot-P/L (in 2003) to the three large municipal treatment plants that serve the city of Stockholm (Stockholm Vatten, 2004). One biological alternative for phosphorus removal is the enhanced biological phosphorus removal (EBPR) process, where the storage capacity of polyphosphate by certain bacteria is utilized (Mino, 1998). The EBPR process demands an alternate operation between aerated and anoxic conditions, and since phosphorus is finally removed from the wastewater through removal of the bacteria the process also produces excess sludge. Polishing, by adsorption in sand filters, is one alternative. At Överjärva, a polishing step for phosphorus removal through the use of microalgae has been tried. The proposed active components are assimilation and pH induced chemical precipitation. The increased pH was due to the photosynthetic activity of algae (Larsdotter et al., 2004). One restriction with this solution is due to the fact that algae need light, and therefore are limited to sunny locations or requires additional light during winter time.

Solar energy has been claimed to be the primary energy source of ecological systems. A significant part of the treatment, however, in a hydroponic system is performed by aerobic bacteria, and aeration is usually the most energy demanding parameter in any biological treatment system. In conclusion, unless aeration is driven by solar power, a substantial amount of energy from other sources is always needed. Solar energy is utilized by plants and algae, and in northern countries natural input is insufficient on a yearly basis. Based on the geographical location, different amounts of additional energy are needed to provide the vegetation with adequate amounts of light and heat for optimal growth (Paper III). As a result, the introduction of green plants to a system rather implies an increased energy demand. However, depending on the value of the products extracted from the system, the additional energy might not be considered merely a cost. Instead, the possibility of producing, "for free", during the summer could be considered to be an advantage for the system. Another option is to prolong the growing season in a greenhouse system during the months when only small amounts of energy are needed to maintain good growth conditions, which can be done without effecting the overall treatment capacity (Paper V). Of course this is purely a hypothetical question, which depends on the probability of finding suitable products for cultivation in an indoor hydroponic wastewater treatment system. Considering energy needed for other applications in the system, such as pumping and internal recycling, the hydroponic system is comparable to other small conventional treatment units, as opposed to the low energy usage by constructed wetlands and infiltration units.

Guterstam and Todd (1990) proposed that ecological systems would produce little or no sludge. In a treatment system, sludge is mainly produced in two ways, through chemical precipitation and bacterial growth. As mentioned before, the micro-biological processes used in hydroponic treatment are basically the same as for other biological systems. In the Överjärva pilot plant, however, very little sludge has been accumulated in the system, and no sludge has been removed or recycled from the clarifier. In theory, the maximum yield constant of aerobic heterotrophic conversion is 0.5 kg biomass/kg organic matter (Henze et al., 2002). Over the years, 2002-2004, around 400 kg of COD has been supplied to the system. Considering the achieved 85-90% removal, an estimated 170 kg of biomass could have been produced in the system during this period. Such a substantial amount should be significant and visible in a small system. One proposed holding step might be the closed tanks, i.e. the anoxic and aerated tanks, although it seems unlikely that this is the only answer. Another probable reason is the long retention time in the system, which should be beneficial for the degradation processes in the system. In principal, the Överjärva system can be viewed as a system with a high sludge age, which would signify a low sludge production (Ødegaard, 1992).

Indications that systems with vegetation produce less sludge, has been reported. In a test train study of planted and unplanted aerated reactors, less than 90 L and over 1140 L of biosolids were removed from the planted and unplanted train respectively during a three months period (Austin, 2000). Their experimental protocol, unfortunately, did not include evaluation of biosolids production and therefore, no further quantitative information is available. In conclusion, a detailed study of biosolids production is required to determine if these systems do produce less sludge than expected, due to the vegetation.

The next short topic concerns the use of chemicals in the system. At Överjärva no chemicals have been added to the treatment process. Chemicals associated with wastewater treatment, however, are mainly related to chemical precipitation of phosphorus, which is a highly effective process. According to the discussion above, it is impossible at this stage to claim that no chemicals should be used until another process has been found that satisfactorily remove phosphorus from the effluent.

Area usage and useful byproducts from a hydroponic system are connected and therefore, these two topics will be discussed simultaneously. One philosophy behind hydroponics is that they are not solely treatment systems, but rather combined treatment and production systems, hereby implying production of green biomass and not energy from digested sludge. If a system is used for production, the area demand is rather defined by the necessary arable area to achieve the expected production capacity. This mainly applies to the aerated hydroponics, i.e. the nitrifying step, and thereby the nitrification process will no longer be the limiting factor for the size of the aerated step.

There are however, a few other very important aspects to consider when wastewater is used as a cultivation medium, i.e. purity and the associated risk factors. Many products demand a very pure medium and wastewater might not be suitable. Although all the nutritional requirements are met, domestic wastewater contain a wide diversity of other substances that will effect the products. To consume products that have been cultivated in wastewater, is probably connected with significant emotional restrictions. In addition, it is not recommended to consume plants that have been grown in hydroponics used to treat primary treated effluents, due to the high concentrations of metals that might have accumulated in the plant leaves (Rababah, 2000). Moreover, uptake and growth of pathogenic bacteria may occur and uptake of virus-sized particles has been demonstrated with lettuce (Rababah, 2000). Hence, edible products can be considered ruled out as potential products. Crops that are beneficial when grown in large areas, such as Salix in wetlands, are also not suitable. The energy to maintain the system in a greenhouse environment would far exceed the possible energy extracted from the system. As I see it, we are left with ornamental plants and cut flowers. The Överjärva system has provided the people working with the system with many beautiful flowers for several occasions during the years. Herein, lies one potential for production in a greenhouse system.

The conclusions drawn based on the **environment** criteria are that a hydroponic system used for production, will use more energy and require a larger area to achieve the same treatment performance as conventional chemical/biological and on-site SBR systems with nitrogen removal (Table 6). Phosphorus removal without chemical precipitation needs to be solved, at least for systems treating high inflow concentrations. Today, it is true that the Överjärva pilot plant uses no chemicals; however, the effluent standard for phosphorus is not reached with the current set-up. The amount of sludge produced in a hydroponic system has not been quantified and therefore it is impossible to relate this indicator to other systems. In constructed wetlands, energy is only used to transport wastewater into the wetlands, thus the energy demand is very low. Compared to hydroponic systems, constructed wetlands require larger areas and the treatment efficiency is lower on a yearly basis.

Table 6. Relative comparison of the environment criteria between hydroponic systems, on-site SBR reactor and constructed wetlands. Results are presented as: (o) comparable to hydroponic systems, (+) more effective or (-) less effective.

	Hydroponics	SBR reactor with chemical precipitation	Constructed wetlands
Treatment performance:			
BOD	o	o	-
Nitrogen	o	o	-
Phosphorus	o	+	o
Use of chemicals	o	-	o
Energy consumption	o	+	+
Area use	o	+	-

Risk aspects related to consuming products have already been discussed; however, there are other **health** issues concerning wastewater treatment such as risk for infections and the working environment. In smaller systems, flows are usually more concentrated due to less in-leakage to the system. In addition, a single source of pathogens is less diluted than in a large system. Results in Paper II showed only slight removal of spores and coliphages in the first two tanks of the system. Safety measures therefore, are recommended regarding human contact with wastewater in the early open parts. The long retention time in the system was beneficial from a pathogen removal perspective, and the effluent quality was comparable to or better than for conventional secondary treatment. Hence, the risk for waterborne outbreaks is relatively small; however, safety measures should also be regarded considering the effluent, and depending on the receiving water body, a disinfection unit might be recommended.

General aspects concerning **technical function** are robustness and the flexibility of the system. Since the hydroponic system is constructed of several smaller modules it might relatively easily be modified according to changing inflows. In a system that include mechanical parts with limited life-times, sooner or later some parts will have to be replaced and that will interrupt the treatment performance. The pilot plant has been subjected to several disturbances during the years. Examples include problems with the air pump, the inflow pump and clogging pipes (Appendix A). The key to robustness is knowledge about how the systems work in the long run and ways to avoid interruptions through adequate construction solutions. I believe that this knowledge exists today and that robust reliable treatment systems, including hydroponics, can be constructed as an alternative to other current on-site systems.

So far, no really significant advantages have been presented in favor of hydroponic systems. Based on the limited potential for recycling nutrients in combination with increased area usage and the dependence on a greenhouse environment in colder climates, it is doubtful that increased sustainability can be claimed for this treatment alternative in Sweden. It is, however, an alternative that can perform the required task, i.e. adequate treatment of wastewater. Further it is performed in an environment which is enjoyable and pleasant. For the local community the greenness of the system might be a fruitful reminder that everything that is fed into the sewer system will eventually end up in nature. The increased transparency of the wastewater system would contribute to public awareness, which in turn could result in less unwanted substances in the wastewater and a healthier environment. Regarding management and costs for the green system, long periods of natural light on a yearly basis is beneficial; therefore, the potential for hydroponic treatment systems in Sweden lies in small decentralized systems, preferably in the southern parts, where the green system and possible products could be utilized by the local community.

CONCLUSIONS

- Treatment of domestic wastewater can be performed in a system combining microbiological processes and hydroponics. Conventional pathways for removal of organic matter and nitrogen applies to the hydroponic system

- Nitrification was performed by bacteria associated to the plants' roots in the hydroponic tanks. The general microbial composition of the biofilm was comparable to other biological systems; however, in relation to other biofilm systems the nitrification rate was low, although sufficient to reach complete nitrification with the current set-up

- The sludge production in the system has been low. Very little sludge has accumulated in the open tanks, and no sludge has been removed from the system. A proposed reason is the high sludge age, which increases the yield for degraded organic matter and biomass in a system

- With an inflow concentration of 15 mg P/L, final polishing for phosphorus removal by means of sand filters and algal tanks was not sufficient. A transition to a larger algal step and no sand filters have been made. Results from the extended algal step will be covered elsewhere. The algal step, however, needs additional light during winter, and therefore other methods to achieve adequate phosphorus removal without adding chemicals to the system need to be investigated as well

- The removal of pathogenic organisms was satisfactory, and the achieved effluent quality comparable to, or better than, for conventional secondary treatment. The long retention time in the system is proposed as one of the reasons for the achieved removal. Safety measures are recommended regarding human contact with wastewater in the early open part of the system

- The area demand for a hydroponic system is larger per p.e. than conventional chemical/biological treatment and small scale treatment plants. If uptake through plant growth and amount of harvested biomass is significant for the system, then this will regulate the size rather than the required volume for sufficient nitrification

- Plants suitable for growth in hydroponic systems have been identified. To date, significant recycling of nitrogen and phosphorus through harvested biomass has not been shown. Valuable plants and other products that will increase the production potential of the system remains to be investigated

- In Sweden, a hydroponic system is restricted to greenhouse applications. To maintain a suitable environment for the green plants, additional energy is needed during dark and cold periods. The necessary amount of energy is related to geographic location. Hydroponic systems therefore, are not recommended as a treatment alternative too far north, unless products are identified that will justify the increased energy usage

- The Överjärva pilot plant has attracted many visitors during the years and thereby contributed to discussions regarding wastewater and treatment. Today's restrictions towards reusing sludge in agriculture are related to the diversity of substances in the inflow to the treatment plants. Some of these substances originate from household chemicals. With green systems, technology can be brought closer to the users, and thus hopefully increase the awareness of the public

- The potential for hydroponic treatment systems in Sweden lies in small decentralized systems, where the greenness of the system and the possible products are considered as advantages for the users, and where these advantages outweigh the disadvantages related to the required greenhouse environment and the increased area demand compared to other current on-site alternatives

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APPENDICES

Appendix A. Överjärva Gård and the pilot plant

Överjärva Gård

Överjärva Gård is an old farm area located in Solna, North of Stockholm. Most of the houses in the area were built in the 19th century, and since 1994 the land and all the houses are owned by Solna town. The pilot plant was constructed inside an old greenhouse, which had been restored between the years, 1996-2000, by applying original construction techniques whenever possible. The greenhouse, which originally is assumed to be from the 1870s, was situated just in front of the main building (Figure 11). During this project, Överjärva Gård was not connected to the municipal water and sewer system. Fresh water was taken from a private well, and before the pilot plant was constructed all wastewater was treated by a sequencing batch reactor (SBR) located beneath the greenhouse.

Figure 14. A drawing of the Överjärva Gård area and the houses within the catchment area (Courtesy of Överjärva Gård AB). Buildings on the photograph are the main building and the greenhouse, summer 2002 (Photo by K. Kauko).

The catchment area included two resident families (5 persons), different shops, the greenhouse and the main building. The main building housed a cafeteria and was also used for biology education for children, local exhibitions and could be rented for other events such as weddings, private dinners or conferences, hence, wastewater load and composition varied throughout the year depending on daily activities. For educational reasons, toilets in the cafeteria were of different kinds, including urine separating toilets and an Aquatron® where solids were separated for digestion elsewhere. Water from the catchment area was collected in a 10 m^3 sedimentation tank and thereafter, stored in an 7 m^3 equalizing tank in the ground just outside the greenhouse. Wastewater to the SBR and the pilot plant was pumped from the equalizing tank.

The sprouting year of 2000

This was the year when it all really began. Starting from the first day of the new millenium, KTH was renting a part of the greenhouse building at Överjärva Gård. Three rooms were included in the package, a 67 m^2 greenhouse for construction of the pilot plant, 20 m^2 for the laboratory and an adjoining room that was going to be the office. What an opportunity!

The first months included a lot of shopping, ordering and construction. Michael Shaw and Paul Zabriskie from Ocean Arks International, USA, were involved in the design of the pilot plant, since they had previous knowledge of constructing similar systems. There are always complications, however, when a house is completed before the interior is planned. In this case, there were restrictions regarding the possible size and format of the tanks. The tanks had to either pass through a common door, or fit in between the wooden frame holding up the glass roof of the greenhouse. When the tanks arrived, one portion of the room was lifted off and finally, all the tanks were inside (Figure 12). The original set-up of the system consisted of one anoxic tank, one aerated closed tank, three hydroponic tanks and one clarifier. The total water volume was 6 m^3.

The educational year of 2001

The first solution for receiving wastewater into the pilot plant was to use the existing pumping system for the SBR. Influent pumping to the SBR was automatic, and determined by three levels. A low-level switch in the equalizing tank stopped the pump and a second level started the pump. When the SBR was full, pumping would cease and the reactor would run its program. The third level was an alarm level, which indicated that the system was over-loaded and the reactor would then run a shorter program, i.e. one aerated sequence instead of two and a shorter sedimentation period. In February, a 300 L tank was installed where a part of the influent wastewater to the SBR could be collected. This water was then used as feed to the pilot plant. In the beginning, wastewater was pumped manually to the anoxic tank, twice per day excluding weekends and holidays. In June, pumping was fully automated and the flow was now delivered every second hour throughout the whole week. An estimated 300 L per day entered the pilot plant. During the summer and autumn, nitrification showed good consistent results with almost complete ammonium reduction already in the first hydroponic tank. In December it was time to experience denitrification, and thus, a recirculation flow was installed from the second hydroponic tank to the anoxic tank.

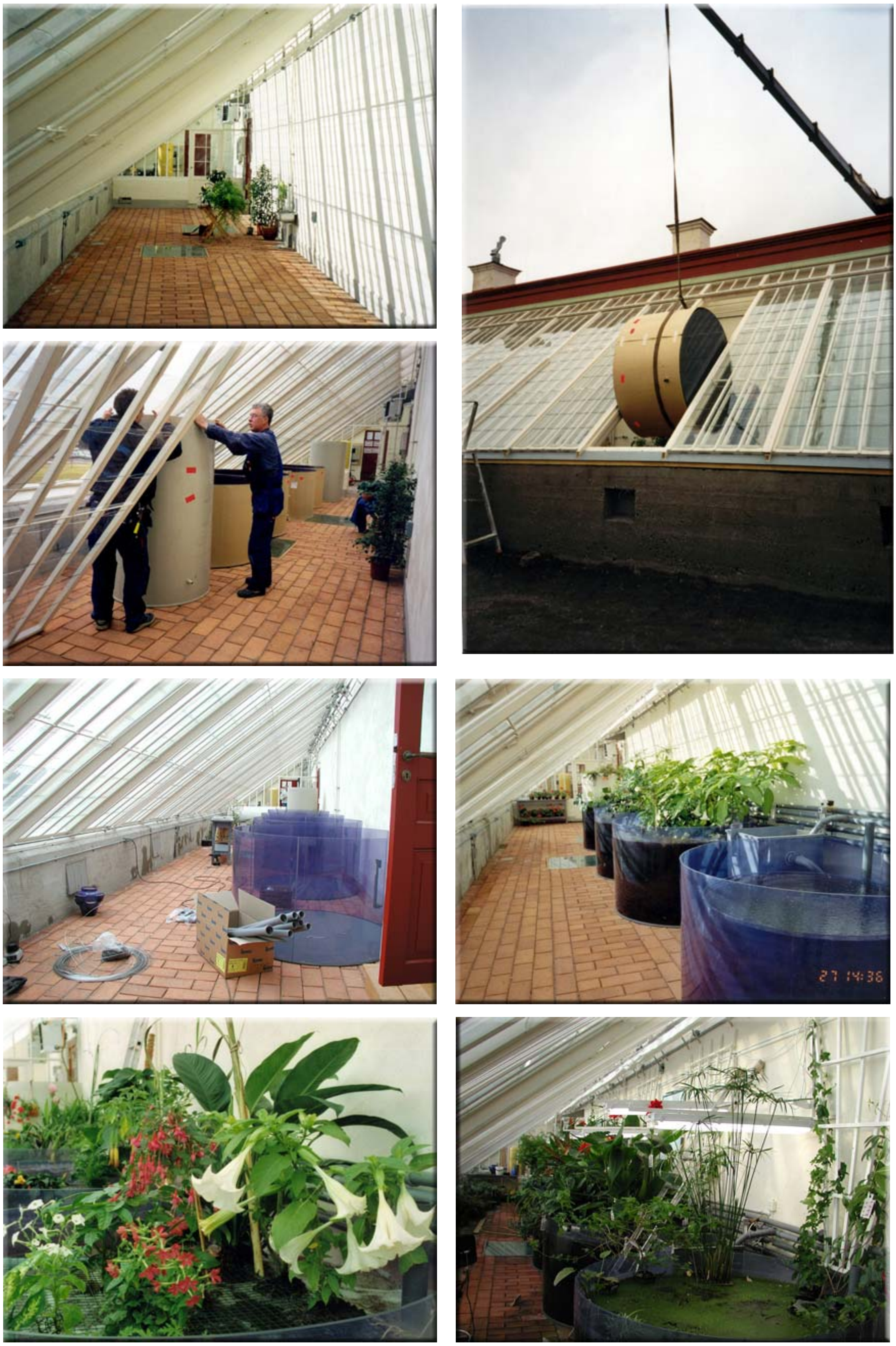

Figure 15. a) January 2000; b) March 2000; c) tank about to land; d) construction site; e) October 2000; f) January 2001; g) January 2002.

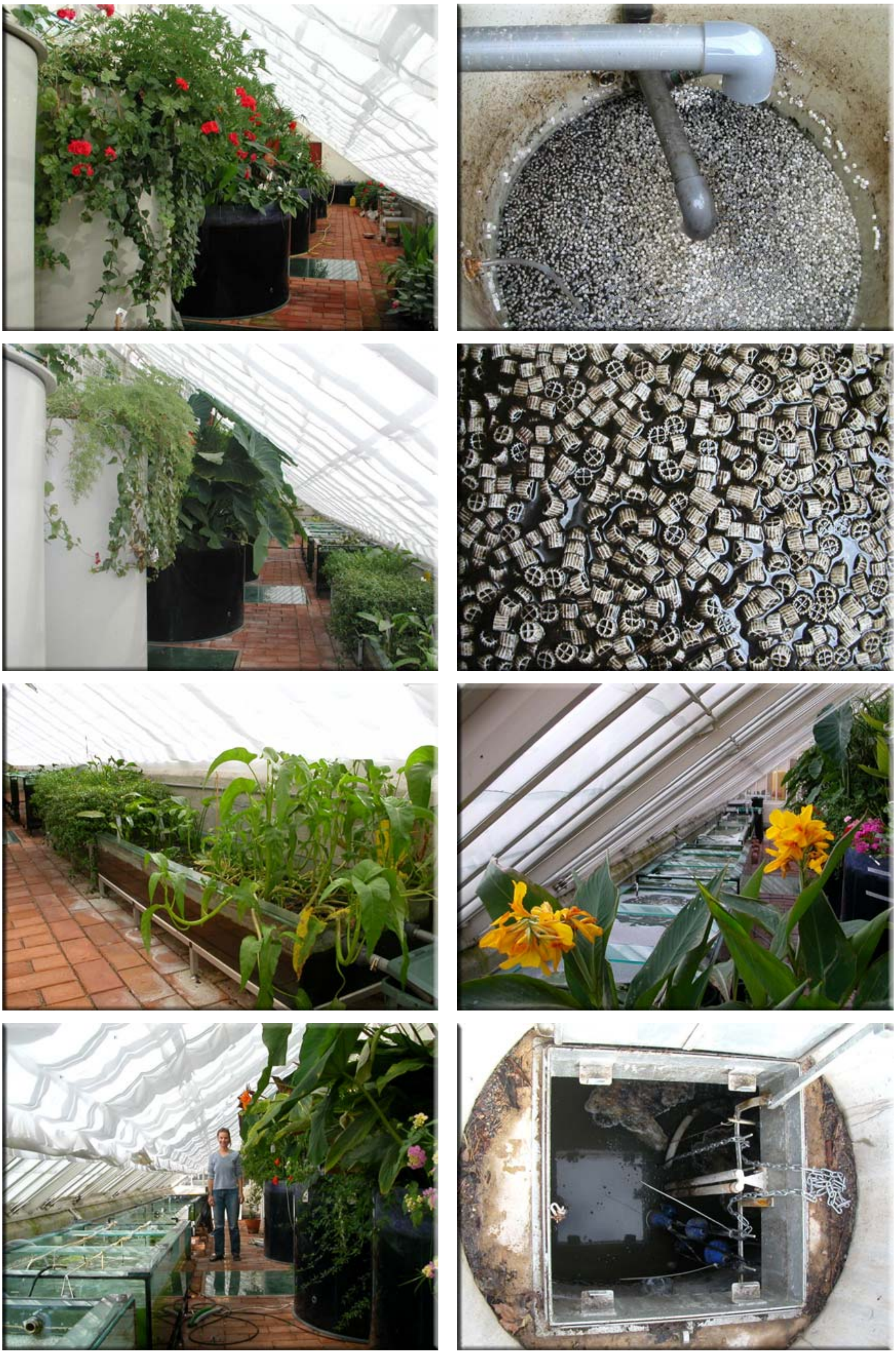

Figure 16. a) November 2001; b) inside the anoxic tank; c) August 2002; d) Kaldnes carriers; e) sand filters; f) increased algal step; g) June 2003; h) the equalizing tank.

Different plants were tried in the system throughout the year to assess their possibility to grow hydroponically. In general, most of the tried plants grew well although they reacted differently to the decrease in temperatures when autumn came. In June, Björn Oliviusson took charge of the management of the green plants and general maintenance of the greenhouse.

The informative year of 2002

After Christmas and New Year holidays, it became obvious that the solution with influent water as a part of the SBR flow did not work satisfactorily. Due to the irregularity of the SBR-pumping and the small volume of the collection tank, most of the wastewater ended up in the SBR system and the collection tank occasionally ran out of water; therefore, another solution was needed. A new pump and a new set of pipes were installed in the equalizing tank and from then on the wastewater was pumped directly into the anoxic tank. In addition, the second level of the SBR-system was raised, which meant that more of the water was available solely for the pilot plant. This solution has proven to work very well over the years.

Due to the availability of more water, the influent to the pilot plant was increased to around 600 L per day and the internal recycle ration was set at around 2. Recycling of oxygen saturated water, however, seemed inhibiting to the denitrification process. As a result, aeration in the hydroponics was adjusted to keep the dissolved oxygen at lower levels while maintaining complete nitrification in the system. Kaldnes carriers were added to the anoxic tank in February 2002, and within a few days both the nitrite and nitrate effluent values from the anoxic tank were low, thus indicating that the denitrification process was working. The system was also extended with new steps to improve the treatment, especially concerning phosphorus removal and final polishing. A sand filter was installed in February and algal tanks in July (Figure 13). The total water volume of the system now increased to 7 m3, and the total hydraulic retention time in the system was 13 days. The microalgae system for removal of phosphorus is part of a separate work and will be presented in detail in the coming doctoral thesis by Karin Larsdotter. Two studies were performed when the system had reached a seemingly steady state. During a three week period in June, the inflow was characterized and treatment performance was evaluated, Paper I. In October, the removal of faecal indicators was investigated, Paper II.

The experimental year of 2003

During this year several changes were made in the system. Heating and additional lights for the green plants were kept at a minimum during the winter period according to the minimum requirements outlined in Paper III. Since there was no cooling system in the greenhouse, however, the indoor temperature was sometimes influenced by the outdoor temperature, i.e. higher than wanted. In the beginning of January, the recycle ratio was increased to around 3. During this period the level of ammonium in the second hydroponic tank slightly increased, probably due to the higher flow, and the recycle pump was moved to the third hydroponic tank. An intensive study, similar to the previous summer study, was performed in February/March.

During summer, the sand filters were removed and the tanks were used for trying phosphorus removal by means of precipitation with Filtralite-P (Optiroc, Norway). The results were a high removal of phosphorus, a large production of precipitated matter that caused problems for the effluent pump and a large increase in pH in the effluent to around pH 12. In conclusion, this method did not work in our system and moreover, the existing tanks were not suitable for further exploring this task. Instead, the tanks were incorporated into the microalgae step where they could be useful. In July, the influent was increased to around 850 L per day. It soon became obvious that the pressure on the anoxic step, i.e. the denitrification, was too large and that the passing time in that tank was not enough. The recirculation flow was decreased and in September, an attempt to expand the anoxic tank was made. Due to several restrictions the solution was an extra tank, which was installed between the existing anoxic tank and the closed aerobic tank. In the original tank, plastic Kaldnes carriers (AnoxKaldnes, Norway) were used, but in the new tank another type of plastic material was introduced to investigate its potential as carrier. After several months of operating with two anoxic tanks, the second tank showed only small tendencies of performing denitrification. Hence, the new carrier material needed to be replaced, or another solution for achieving denitrification with the higher flow was needed for the process to work as efficiently as before.

The microbiology year of 2004

With a somewhat clear idea of how the system functioned and the processes involved, time had come to look deeper into "who is really doing the job". The method of choice was fluorescent *in situ* hybridization (FISH). With a battery of different probes the microbial flora of the first hydroponic tank was investigated during a six months period. The FISH study was performed during a stay at Technische Universität München (TUM) in Freising, Germany, and the results are presented in Paper VI.

Unwanted experiences

or "There is no such thing as a free lunch". The ultimate dream system should work without any interruptions; however, in the real world unexpected events occur to teach us more about things we did not know we wanted to know.

Wandering carriers. The anoxic tank contains Kaldnes carriers (Figure 13) and without attached biofilm, these carriers float. Following inflow pumping, the carrier surface always slightly rose above the water surface. Usually this was counteracted by pushing the carriers back into the water, approximately twice per week. The gas filled spaces of the anoxic tank and the closed aerobic tank was connected, and occasionally a few carriers would move on in the system. At one occasion, enough carriers had stayed in the pipe between the closed aerobic tank and the first hydroponic tank to block the water flow. Today, plastic nets are covering all the exit holes from the anoxic tank.

Malfunctioning air pump. The air pump to the system was located in the SBR room to keep the noise level down in the greenhouse. Air is not always as clean as one might expect, and without an effective filter, the pump will eventually clog, with a subsequent loss of pump head. The result is, of course, inadequate aeration and decreased treatment capacity. The air pump is now equipped with a beautiful mushroom-like filter and has been working without any further disturbances for over three years.

Crashing soil filter. The cover on the closed aerobic tank was a planted soil filter made up of compost/sand and shredded bark lying on a fabric layer. The filter was built on top of a stand made from plastic pipes and a plate of glass fiber. During the summer 2003, one of the stand's legs tilted and the filter crashed down into the tank. Karin spent two days remaking the filter while I was on vacation.

Overflow in the collection system. One sunny summer day, the ground outside the greenhouse was wet and there was an unmistakable smell of wastewater in the air. The water had leaked from overfilled inspection points. The reason turned out to be a sludge plug between the collection tank and the equalizing tank. A hero in the form of a driver of a sludge suction truck, emptied the collection tank, removed the plug and saved the day and our inflow.

Washout. A small catchment system is easily influenced by its users. One day the inflow analysis gave very strange results, the wastewater had basically turned into water. The villain was an open tap located in one of the buildings that is not normally used. Probably someone had tried to use the tap during the annual Christmas market while the water in the pipe was frozen, and then left the tap open. The result was a fresh water spring flood in our system. It took several weeks before the inflow to the system showed normal characteristics again. This was probably the most unwanted experience of them all, considering that it occurred while I was in the middle of the sampling period for the FISH-study. For the sake of the study, however, it turned out to be educational.

Broken inflow pump. After almost three years of faithful service, the pump located in the equalizing tank gave up and was replaced.

Appendix B. Fluorescent *in situ* hybridization

Hybridization, in general, depends on the ability of denatured DNA to reanneal with complementary strands in an environment just below their melting point. During *in situ* hybridization, a small fragment of DNA or RNA anneals to a specific target strand of DNA or RNA in a morphologically preserved cell (Amann et al., 1990). Under the right conditions, this fragment or probe, will bind to a specific genetic sequence within that cell. If the complementary sequence or match is not present, hybridization does not occur and the probe is washed from the cell.

For determinative studies of microorganisms, ribosomal RNAs have proved to be well suited (Stahl and Amann, 1991). They are highly conserved biopolymers, including great variation in regional sequence conservation. Based on previous identified sequences, synthetic oligonucleotides can be tailor made to hybridize to specific sequences. Depending on the probe design, the target may be as specific as a single organisms or as broad as all prokaryotes. To detect probes after hybridization, reporter molecules must be attached, and in this study fluorescent dye labels were used. The high copy number of generally many thousands of rRNA per cell, gives greater sensitivity to the detection of the reaction product. Due to the high copy number, sufficient dye can be incorporated in the cells to be visualized by fluorescent microscopy (Stahl and Amann, 1991). Since probes can be marked with different fluorescent dye molecules, several levels of identification can be viewed simultaneously in the same sample (Figure 15).

The hybridization procedure includes exposing cells to elevated temperatures, detergents and osmotic gradients. Therefore, fixation is essential to preserve the morphology of the cells throughout the procedure. In addition, fixation serves to minimize endogenous nuclease activity and other degradation processes, and should be performed soon after sampling (Leitch et al., 1994). Two fixation protocols have been used in this study; paraformaldehyde fixation for gram-negative bacteria and ethanol fixation for gram-positive bacteria. Paraformaldehyde acts as a cross-linked fixative and the fixed cells exhibit good morphology and good retention of target sequence (Leitch et al., 1994). Probe permeability of paraformaldehyde fixed cells of certain gram-positive bacteria is often limited (Amann, 1995), and therefore, ethanol fixation was used in parallel on all samples. Fixation samples can be stored in a -20°C freezer for months when ethanol is added, to prevent the samples from freezing. Before hybridization, samples can be dehydrated in en ethanol series and subsequently air dried. Although this step is not essential, it ensures that the probes are not diluted by any residual pre-hybridization solutions (Leitch et al., 1994).

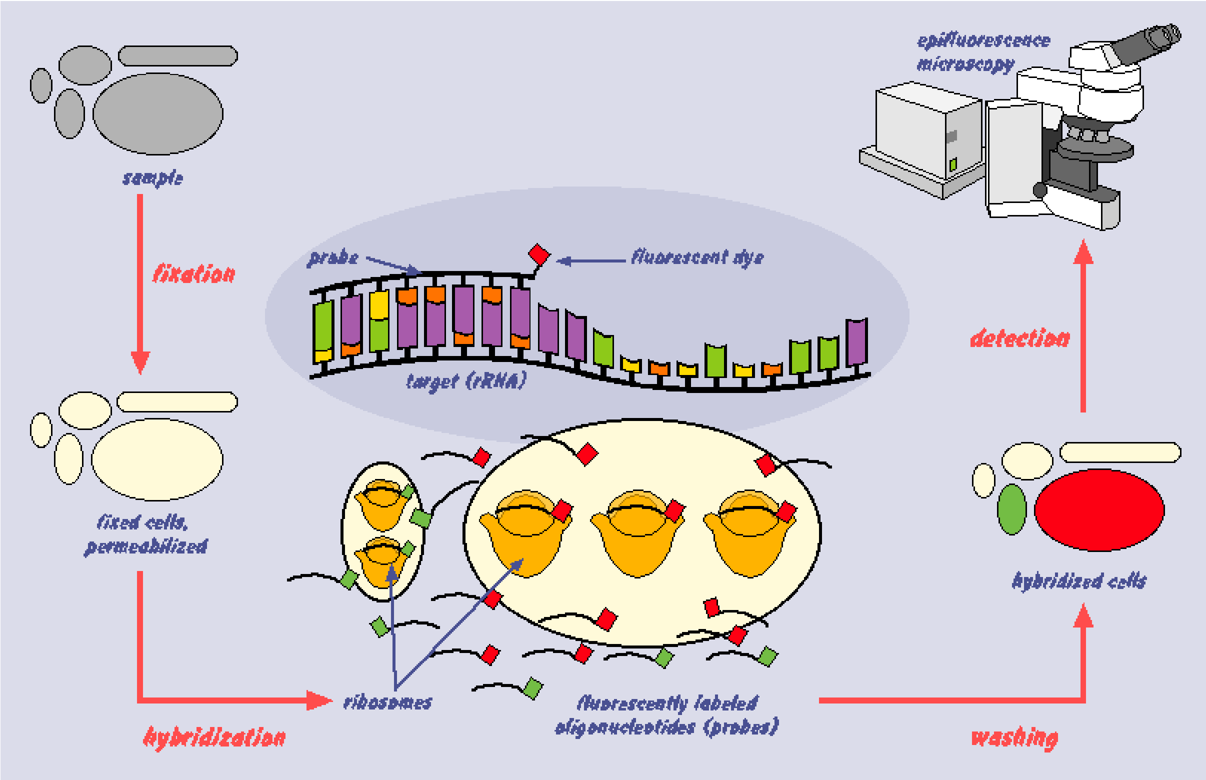

Figure 15. A schematic overview of the steps included in the FISH procedure.

Important considerations for nucleic acid hybridization include specificity and stringency. Oligonucleotide probe specificity is determined by its length and the complexity of the target sequence. For determinative studies of cells extracted from the environment, probes are usually 15-25 nucleotides in length. How well a given set of hybridization conditions control probe specificity is more commonly referred to as, the hybridization stringency. Stringency, or mismatch discrimination, is controlled by temperature, ionic conditions and presence of organic solvent in the hybridization and post-hybridization wash solutions (Stahl and Amann, 1991; Amann et al., 1995).

Hybridization will not occur in the absence of salts, due to repulsion between the negatively charged phosphate groups in the nucleic acids. Ionic conditions are often monitored with monovalent cations, e.g. sodium ions. These cations interact with nucleic acids and decrease the electrostatic repulsion between the two strands, thereby increasing the stability of the hybrid. Organic solvents are used to reduce the thermal stability of the double-stranded nucleic acids and thus decrease the temperature required for dissociation. Heating a solution of double stranded DNA unravels the duplexes by disrupting the hydrogen bonds that pin together complementary base pairs. Normally, temperatures of 65°C and above are necessary to maintain a single stranded target sequence, which will cause thermal degradation of nucleic acids over time, especially of RNA. For hybridization, a common destabilizing molecule is formamide, which disrupts the hydrogen bonds in the double helix. The addition of formamide to the hybridization solution lowers the melting point of the double helix by about 0.6°C for every 1% added to the hybridization buffer (Stahl and Amann, 1991).

As a result of added formamide, hybridization can be performed at lower temperatures without loss of high stringency. For individual probes, the concentration of formamide in the hybridization solution is experimentally determined, since the rules for predicting stability of the secondary or higher structures are only approximations (Stahl and Amann, 1991). In addition to formaldehyde, sodium dodecyl sulfate (SDS) is commonly used. SDS is a strongly anionic detergent that helps to linearize the nucleic acid strain and thus make it more accessible to hybridization. Currently, most hybridization work is being performed at 46°C and formamide and salt concentrations are varied instead (Daims et al., 2001; Lee et al., 2003; Schmid et al., 2000; Wagner et al., 2002).

Conditions for high stringency are optimized to assure that only probes with high similarity to the target sequence will be stable enough to remain hybridized (Leitch et al., 1994). An ideally designed oligonucleotide probe should form a perfect duplex only with its target sequence. During hybridization, however, duplexes form between perfectly matched, as well as some imperfectly matched, sequences. This background hybridization of probes nonspecifically bound to nontarget sequences is removed in the post-hybridization wash step; thus, stringency is adjusted in one or both of the hybridization and the post-hybridization wash steps (Stahl and Amann, 1991).