NITROGEN TRANSFORMATIONS AND REMOVAL MECHANISMS IN ALGAL AND DUCKWEED WASTE STABILISATION PONDS

Nitrogen Transformations and Removal Mechanisms in Algal and Duckweed Waste Stabilisation Ponds

DISSERTATION
Submitted in fulfilment of the requirements of
the Academic Board of Wageningen University and the Academic Board
of the International Institute for Infrastructural, Hydraulic and
Environmental Engineering for the Degree of DOCTOR
to be defended in public
on Tuesday, 25 March 2003 at 15:00 h in Delft, The Netherlands

by

Omar Zimmo
born in Gaza, Palestine

This dissertation has been approved by the promoter and co-promoter:
Prof.dr. H.J. Gijzen, IHE Delft, The Netherlands
Dr.ir. N.P. van der Steen, IHE Delft, The Netherlands

Members of the Awarding Committee:
Prof.dr. D.D. Mara, University of Leeds, United Kingdom
Prof.dr.ir. A.J.M. Stams, Wageningen University, The Netherlands
Prof.dr. P. Denny, IHE Delft, The Netherlands
Dr. R. Al-Sa'ed, Birzeit University, Palestine

Published by: A.A. Balkema Publishers, a member of Swets & Zeitlinger Publishers
www.balkema.nl and www.szp.swets.nl

ISBN 90 5808 811 1 (Wageningen University)

Dedicated to

my Mother,
my Wife, Suha,
my children, Basil, Iyad and Tala

God bless them all

ABSTRACT

This thesis describes the results of a comparative study of the performance of algae-based ponds (ABPs) and duckweed-based ponds (DBPs) for wastewater treatment, with emphasis on nitrogen transformations and removal mechanisms.

Batch experiments simulating algae and duckweed (*Lemna gibba*) stabilisation ponds for domestic wastewater treatment were conducted to quantify the importance of various nitrogen removal mechanisms under controlled conditions of pH and DO. N-removal in both systems by different mechanisms are more dependent on the pH variations than on the oxygen variations. Significantly higher N-removal efficiency in the duckweed system (26-33%) than in the algae system (14-24%) was found at lower pH range of 5 to 7. At high pH values of 7 to9, the increase in N-removal by sedimentation and volatilisation in the algae system and the decrease in N-uptake by duckweed in the duckweed system resulted in significantly higher N-removal efficiency in the algae system (45-60%) than the duckweed system (38-41%).

Further research was carried out in pilot-scale algae-based and duckweed-based systems that consisting of 4 similar ponds in series for each system, fed with wastewater with hydraulic retention time of 7 days in each pond.

Tracer experiments showed that the hydraulic characteristics were similar in both ABPs and DBPs. Actual retention times were observed to be higher than the theoretical retention times due to the spurious tracer curves resulting in negative dead spaces. This suggests that the higher density of LiCl solution compared to pond wastewater density, probably caused LiCl to pass onto the bottom of the pond and slowly dissolved into pond water where it leached out at a much longer period and thus giving spurious tracer curves. The hydraulic behaviour of the ponds was neither plug-flow nor completely mixed, but rather showed a dispersed flow. A tracer experiment in larger scale algae and duckweed-based ponds in Colombia showed lesser short-circuiting and more plug flow conditions in the duckweed pond than the algae pond. For larger surface areas the presence of duckweed cover improved the hydraulic performance of pond. The better hydraulic behaviour of the pond with duckweed cover may be explained by reduced wind-induced short circuiting and reduced mixing caused by the absence of algae biomass activities.

Higher BOD and TSS removal efficiencies were achieved in DBPs compared to ABPs. In both systems, the removal of BOD and TSS did not differ significantly during the different seasons of warm and cold weather. Total-P was more effectively reduced in DBPs than in ABPs, irrespective of the season due to phosphorus uptake of duckweed and subsequent removal from the system via harvesting.

Increase in organic loading resulted in an increase in BOD and TSS removal rates in both ABPs and DBPs. Phosphorous removal was similar during the two experimental periods in ABPs. DBPs behaved likewise. Removal of FC in ABPs was higher than in DBPs. FC removal in the ABPs and in the DBPs was significantly higher during low organic loading period compared to high organic loading period. FC removal in ABPs during the low and high organic loading periods was 3.8 and 3.4 log units, respectively. Corresponding values for DBPs were significantly lower at 2.2 and 1.8 log units. Lower FC removal was found during the cold period in both systems.

During the low organic loading period at warm temperature, nitrogen removal efficiency was higher in ABPs (80%) than in DBPs (55%) despite the fact that approximately one third of the influent nitrogen to the DBPs is removed via duckweed harvesting. Lower N-removal in both systems was obtained during cold season but similar removal was found during periods of low and high organic loading.

Quantification of N-fluxes in both systems showed that the major fluxes of nitrogen in the ABPs were sedimentation (33-40%) and denitrification (14-24%). Sedimentation and denitrification in DBPs were of equal importance except during the warm season and low organic loading operation, when sedimentation was low. In DBPs, 30-33% and 15% of the total nitrogen was recovered into biomass and removed from the system via duckweed harvesting during the summer and winter period, respectively. During the high organic loading period, nitrogen recovered via duckweed was 19%. Ammonia volatilisation in both treatment systems was found to be a minor N-removal mechanism responsible for less than 1.1 % of total influent nitrogen. Nitrification and denitrification occurred in the aerobic water phase and anaerobic sediment, respectively. Higher DO concentrations in ABPs, especially during the warm season, favoured higher nitrification in ABPs as compared to DBPs. Predictive models for nitrogen removal in ABPs and DBPs were proposed. They presented a good reflection of nitrogen fluxes on overall nitrogen balance under the prevailing experimental conditions. Validation of the models with reported data from literature gave poor results for shallower ponds, while better agreement was obtained using data for deeper ponds. Further elaboration and validation of the model to accommodate pond design and environmental parameters that dictate pond performance is required.

CONTENTS

Chapter 1

INTRODUCTION

INTRODUCTION

CHALLENGES IN THE WATER SUPPLY AND SANITATION SECTOR

There have been substantial developments in (waste) water management and treatment technology during the past decades (Gijzen, 2001a). In spite of that, in 1997 three billion people on earth lacked adequate sanitation. In Africa alone, 80 million people are at risk from cholera, and 16 million cases of typhoid infections each year are a result of lack of clean drinking water and adequate sanitation (WHO, 1996). Approximately 95% of the generated wastewater in the world is released to the environment without treatment (Niemczynowics, 1997). Wastewater has been identified as the main land based point source pollutant causing contamination of the (coastal) marine environment (UNEP/GPA, 2000). The increase in population and therefore in sewage production imposes a great challenges to develop and introduce sustainable sewage collection and treatment systems in many countries in the world. The efforts in providing these essential services especially for poorer regions of the world are hindered by the shortcomings of the current concept of urban water management and financial limitations.

Current concept of Urban Water Management

The technology development for urban water management was based on the following concepts (Gijzen 1999; Harremoës, 2000): a- the prevention of water-borne diseases, b- the provision of large quantities of high quality water within a supply-driven system and c- the use of water to transport waste out of the city. These concepts have resulted in misuse of water in urban areas. Some examples of misuse are the use of drinking water quality for cleaning cars, watering gardens and flushing toilets, the use of water consuming devices for washing (dishes, clothes, shower) and sanitation (flush toilet) and the use of 50 to 80 litres of high quality drinking water to transport 1-1.5 kg of human waste to a wastewater treatment facility. After disposal into the sewer this combined waste is mixed further with industrial effluents, and in some occasions with urban runoff. With these commonly used practices, less polluted water is polluted and strongly polluted wastewater is diluted, thereby creating expensive transport of large amounts of waste which have to be treated and disposed off (Bergen, 1997). These practises in water uses also make re-use of specific components in the mixed and diluted waste flow less feasible. One and a half century after the introduction of the current urban water management, it is high time to address the shortcomings of the current concepts of urban water and sanitation services.

Costs of sanitation infrastructure systems

Providing the necessary funds for capital cost and operation and maintenance cost is a key issue for development of the sanitation sector especially in low income regions. Grau (1994) and Gijzen and Ikramullah (1999) calculated the period of time required to generate the capital investments to meet EU effluent standards in low GNP countries and found this period to exceed by far the economic life span of treatment plants and sewer infrastructure. World Bank studies (1982) on the cost for water supply and sanitation for a wide variety of technology options, concluded that the costs for sanitation systems is five to six times higher than the cost of drinking water supply. Gunnerson and French (1996) demonstrated that the ratio disposal/supply costs increases further when service levels and water consumption increase (Figure 1). Willingness to pay for sanitation infrastructure is generally lower than for water supply services. This fact, together with the higher costs per unit of volume explains the lower level of success in improving sanitation coverage compared to water supply. This demonstrates the complexity and great challenges to develop and introduce sewage collection and treatment systems and it explains the small total volume of wastewater that receives treatment world-wide.

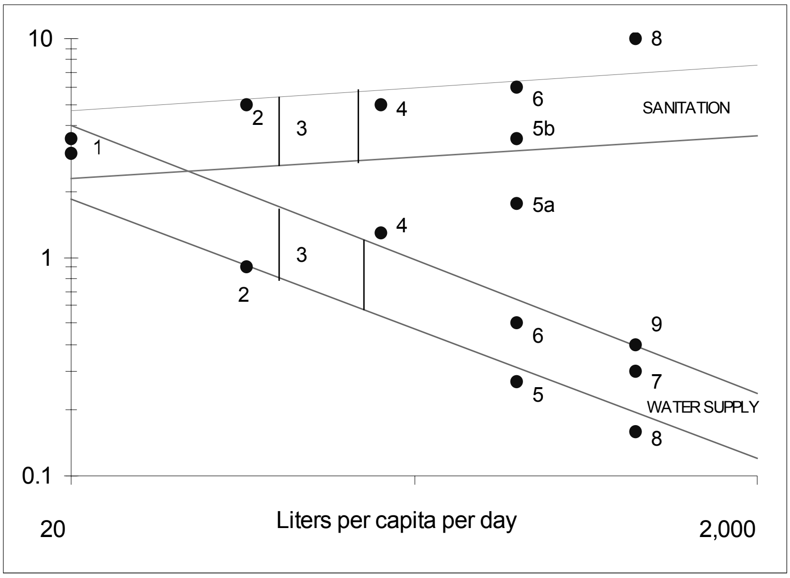


Figure 1. Water consumption versus costs for water supply and sanitation systems (Gunnerson and French, 1996). Costs include distribution/collection and treatment. Numbers refer to: 1) Village scale hand pump and household latrines, 2) Jakarta, 3) World Bank basic needs level, 4) Malace, Malaysia, 5) Kyoto, Japan (5a =household vault and vacuum truck to truck sewer; 5b = conventional sewer), 6) Washington, 7) Boulder, Colorado, 8) Chicago, 9) Los Angeles

WASTEWATER MANAGEMENT CONCEPTS

The current concept for wastewater management is based on looking for solutions and interventions exclusively 'at the end of the pipe'. There are a variety of technologies available to substantially reduce organic compounds and nitrogen levels in municipal sewage. Current wastewater treatment such as activated sludge systems, with tertiary nitrogen removal, are too costly to provide a satisfactory solution for the increasing wastewater problems in developing regions (Gijzen, 2002). These technologies do not allow for reuse of valuable energy and nutrients present in the wastewater. In the light of these limitations, the current wastewater management strategies should be reconsidered (l litre of water used = 1 litre of wastewater produced). Gijzen *et al.* (in prep.) proposed a better approach for wastewater management in which final discharge is optimised in a three stage approach. The first stage takes into consideration the concepts of pollution prevention starting at the household level. The second stage deals with wastewater treatment. New technologies that aim at a wider context of pollution prevention, nutrient utilisation and water reuse are recommended. The third stage is at the effluent discharge level, which aims at optimal utilisation and/or boosting of the self-purification capacity of receiving water bodies.

Pollution prevention

The current urban sewage treatment and management practises should be reviewed critically in order to limit pollution spreading world-wide. This could contribute to the conservation of ecosystems, which is defined as one of the key issues for Vision21 and the world water vision that was presented during the second World Water Forum in March 2000 in The Hague (Cosgrove and Rijsberman, 2000). The minimisation of water use and waste separation will be the key issues to be addressed by any intervention in this respect (Mahmoud, 2002).

Minimising wastes at the source

A waste minimisation approach aimed at reducing water consumption could yield substantial savings, both at the supply end as well as on the sanitation end of the pipe (Gijzen, 2001a,b). Per capita consumption could drop due to awareness raising, demand management schemes and via the introduction of water saving technologies (low or no-water sanitation devices). It is expected that such strategy needs to be adopted due to increase in population and decrease in water resources especially in regions with scarce water resources. For the long term, low water use or even dry sanitation practises may be introduced in combination with modern collection (vacuum systems) and anaerobic composting processes (Gijzen, 2002).

Waste separation and reuse concept

The separation of black and grey wastewater at household level and their onsite treatment is a rational option, which contributes to the sustainability of wastewater management (Lettinga *et al*., 2001). This approach is useful in order to optimise the potential for re-use. It starts at the level of the household, where grey water could be re-used for toilet flushing or garden irrigation. Besides the separation of grey and black water, also urine could be collected separately for recovery of N and P fertiliser (Larsen and Guyer, 1996).

Treatment and resources recovery

In line with the "Vision 21" for developing regions, the reuse concept, energy and nutrients recovery and water reuse are components that should be included in planning and development of sustainable innovative sewage treatment technologies. Following some of the current wastewater technology concepts and reuse strategies and possible interventions are presented, which could make wastewater management more affordable and sustainable.

State-of-the-art of wastewater treatment technology

At present aerobic treatment systems are the most commonly used technology for wastewater treatment. These systems involve the energy-intensive supply of air or even pure oxygen for oxidation of organic matter. Nitrogen removal is realised in these systems via enhancement of biological nitrification via additional oxygen input and

subsequent denitrification under anoxic conditions in order to strip nitrogen from the water and convert it into atmospheric N_2 gas.
Due to a rapid increase in energy costs, anaerobic treatment gained more attention over the past 30 years. Developments include the anaerobic filter (AF) (Young and McCarty, 1967), the upflow anaerobic sludge blanket reactor (UASB) (Lettinga *et al*., 1980), the fluidised and expanded bed reactors (Switzenbaum and Jewell, 1980) and the downflow stationary fixed film reactor (Murray and van der Berg, 1981). Anaerobic treatment has been proposed as the central unit of sustainable waste management (Gijzen, 2001a; Hammes *et al*., 2000; Mahmoud, 2002; Zeeman and Lettinga, 1999).
Pathogens can be removed efficiently by simple and effective methods using stabilisation ponds (Pearson *et al.*, 1995; Shuval *et al.,* 1986). With this system it is also possible to remove nutrients (Silva *et al*., 1995). Conventional stabilisation ponds fit in the reuse principle since the effluent can be used for irrigation.
Constructed wetland systems are also low cost treatment technology, which are within the economical and technological capabilities of most developing countries.

Limitations and drawbacks of wastewater treatment technology
Aerobic treatment systems are usually sophisticated and expensive, since substantial input of external energy is consumed. Therefore, its application to sewage treatment in countries with a low GNP will be limited. Besides, this approach in wastewater treatment does not allow for reuse of valuable energy and nutrient resources contained in wastewater.
Although the conversion of organic matter by anaerobic treatment systems in high quality biogas where some 90% of the energy contained in organic matter, so far no commercial amount of biogas from wastewater treatment has been developed. Reduction of nutrients (N and P) and pathogens in anaerobic reactors is minimal. An additional post-treatment therefore is required.
Generally waste stabilisation pond (WSP) systems are considered to be ineffective in nitrogen removal, since one form of nitrogen is converted into another. The nitrogen incorporated in algae (typical formula $C_{106}H1_{81}O_{45}N_{16}P$) is temporarily stored due to sedimentation in the final stages of the pond but subsequently remineralised. The non-settled part of algae biomass will leave the system with the effluent. In most cases the appropriate nitrogen concentration for agricultural use will not be realised. Algae in the effluent represent also BOD load. Assuming 50% removal efficiency of total nitrogen in the stabilisation ponds, an average concentration of 60 mg-N/l of total nitrogen in sewage may lead to the production of almost 300 mg/l (dry weight) algae biomass, which is equivalent to or higher than the original BOD present in sewage. The presence of algae biomass in pond effluent represents one of the causes of emitter clogging in drip irrigation systems (Bucks *et al*., 1979). The direct effect of algae biomass in eutrophication, makes waste stabilisation pond systems in need of further development.
In constructed wetlands, plants used (e.g. Phragmites and Typha) can reach 3 m in height, which complicates harvesting management. Besides, the high fibre content of the plants used makes it unsuitable to be used as animal feed. Moreover, a wetland treatment technology seems inappropriate in areas of scarce water resources due to high water losses via evapotranspiration in these systems.

Reuse of water and nutrient recovery
The reuse of raw wastewater for irrigation is a global practice world-wide. Wastewater is essentially good for irrigation since its nutrient content (NPK) may result in substantial reduction or even complete replacement of commercial fertilisers. However,

pathogens contained in wastewater make it unfavourable for reuse due to associated risks. Also, excessive amounts of nutrients in water might be detrimental to plants (Mujeriego and Asano, 1991). Therefore, it is urgent to develop a treatment technology within the economical and technological capabilities of developing countries which aim at reducing the nitrogen level to optimal value that match crop requirements and produce an effluent with low TSS, BOD and pathogens. Recovery of nutrients from wastewater that can later be reused could be enhanced by increasing the incorporation efficiency of nitrogen into plant protein, by the use of duckweed in wastewater treatment. Duckweed stabilisation ponds are modified waste stabilisation ponds, covered with a floating mat of aquatic plants (*Lemnaceae*). The duckweed harvested at regular intervals can be used to cultivate fish in adjacent ponds, while the effluent can be made available for irrigation (Gijzen, 2001b). Promising results from a combined system of duckweed ponds and fish ponds has been realised in Bangladesh. The pond complex receives wastewater from 2000-3000 capita at hydraulic retention time of 21 days. The results demonstrated that the combined wastewater and fish aquaculture system produced over 12 tons fish/ha/year, yielding a net annual profit of about US$ 2000/ha (Gijzen and Ikramullah, 1999; ICDDRB, 1995). Also, preliminary epidemiological monitoring at the site provides evidence that the use of harvested duckweed in fish production does not induce a risk of pathogen transfer (ICDDRB, 1995). If we succeed to convert the nutrients in wastewater into valuable protein, this will reduce the need for further treatment for tertiary removal of nutrients, reduce the use of commercial fertilisers, improve food security and save the scarce fresh water for other user functions.

Self purification capacity

Having achieved waste minimisation and efficient wastewater treatment, which utilises energy and nutrient recovery, the third measure would be to boost natural self-purification of receiving water bodies. Enhancement of self-purification capacity in receiving water bodies could reduce the environmental impact of wastewater or effluent discharges. This could be achieved via good knowledge of the purification capacity and to boost it via proper engineering design. A good example of boosting the purification capacity is the Cienaga de la Virgen, a natural lagoon in Cartagena, Colombia (Gijzen *et al*., in prep.).

It is not realistic to expect a major intervention in the current concepts and strategies on the short run as substantial changes in the current water supply and sewage infrastructure are needed. The complexity of interrelated aspects in connection with water use and wastewater disposal makes changes in this sector more difficult. Great investments in household and municipal infrastructure are needed. In order for changes in the water sector to be realised, staged changes in traditional approaches should be adopted. Meanwhile, current strategies for water saving, recycling, use of non-conventional water for irrigation and enhancement of cleaner technologies should be implemented.

WASTEWATER TREATMENT BY MACROPHYTES-BASED PONDS

The use of aquatic macrophytes

Aquatic macrophytes have been suggested as a low cost option for the purification of wastewater and production of plant biomass (Araujo, 1987; Brix and Schierup, 1989; Gijzen, 1996; Oron, 1994; Reddy and DeBusk, 1987; Skillicorn *et al.,* 1993). Water hyacinth *(Eichhornia crassipes),* pennywort *(Hydrocotyle umbellata),* water lettuce *(Pistia stratiotes)* and duckweed *(Lemnaceae)* have been reported for the efficient removal of nutrients (Gijzen, 2001b). Water hyacinth has been used most widely, due to its high nutrient uptake capability, but no attractive application of the plant biomass has been identified so far. The use of duckweed in wastewater treatment is not well documented, nor are design criteria available. The scarce studies reported so far suggest that this technology holds great promise as low cost wastewater treatment.

What is duckweed?

Duckweed is a floating aquatic macrophyte belonging to the botanical family *Lemnaceae* which can be found world-wide on the surface of nutrients rich fresh and brackish waters. The *Lemnaceae* family consists of four genera (*Lemna, Spirodela, Wolffia* and *Wolfiella*) and 37 species have been identified so far.
Compared to most other plants, duckweed has a low fiber content (about 5 %), since it does not require structural tissue to support leaves and stems. The nutrients taken up by duckweed are assimilated into plant protein. Under ideal growth conditions protein content of more than 40 % on dry weight basis may be achieved (Oron *et al.*, 1985; Landolt, 1986; Skillicorn *et al.*, 1993). Duckweed protein has relatively high concentrations of the amino acids lysine and methionine resembling animal protein. Trace minerals and pigments are also present in high concentration, which make duckweed favourable as nutritious animal feed. The efficient uptake of nutrients such as N and P from the water body could be applied for removal of nutrients from wastewater. Different studies have shown that duckweed systems are capable of treating wastewater (Alaerts *et al*., 1996; Reddy and DeBusk, 1985).

Duckweed ponds for sewage treatment

Wastewater treatment by duckweed covered ponds has attracted the attention of researchers in various parts of the world. Duckweed-based pond (DBP) systems have been applied at full-scale in Taiwan, China, Bangladesh, Belgium and the USA (Edwards, 1980; Zirschky and Reed, 1988; Alaerts *et. al.*, 1996). Solids and organic materials in the wastewater are removed via sedimentation and bacterial mineralisation, while the plants are active in nutrient removal. DBP systems might have the following advantages over WSP systems:

- The duckweed cover restricts sunlight penetration into the water body, and this limits algae development. Markedly lower TSS levels are therefore expected in the final effluent (van der Steen, 1998).
- Evapotranspiration is lower than evaporation from an open water surface under the same meteorological conditions (Oron *et al.*, 1985; Oron, 1990).
- Duckweed system may generate economic return via the commercialisation of biomass for fodder and effluent for irrigation (Skillicorn *et al*., 1993).

Performance of duckweed-based pond systems in N-removal

Nitrogen uptake by duckweed

Duckweed prefers ammonium to nitrate as a source of nitrogen (Porath and Pollock, 1982). Lüönd, (1980) reported 10-20% higher biomass yields when duckweed was grown on a medium containing NH_4^+, compared to growth on $NO3^-$. Values on daily nitrogen uptake by duckweed are shown in Table 1.

Table 1. Nitrogen and phosphorus uptake by duckweed.

Region	Species	Daily removal ($g/m^2.d$)		Reference
		N	P	
Louisiana	Duckweed	0.47	0.16	Culley *et al.* (1978)
Italy	*L.gibba/L.minor*	0.42	0.01	Corradi *et al.* (1981)
USA	*Lemna*	1.67	0.22	Zirschky and Reed (1988)
India	*Lemna*	0.5-0.59	0.14-0.30	Tripathi *et al.* (1991)
Minnesota	*Lemna*	0.27	0.04	Lemna corporation
Florida	*Spirodela polyrrhiza*	-	0.015	Sutton and Ornes (1977)
CSSR	Duckweed	0.2	-	Kvet *et al.* (1979)
Bangladesh	*Spirodela polyrrhiza*	0.26	0.05	Alaerts *et al.* (1996)
Yamen	*Lemna*	0.05-0.2	0.01-0.05	Al-Nozaily (2001)

The wide range in the data presented in Table 1 is explained by differences in duckweed species, (waste) water composition, nutrient concentration and climatic conditions. A major advantage of DBP is that N-incorporated in duckweed can be easily removed via harvesting, and in this way contribute significantly to overall N removal from the wastewater.

Overall nitrogen removal

Nitrogen uptake by duckweed has been studied for different types of wastewater at laboratory scale, as well as in outdoor experiments and these experiments have shown the potential of duckweed-based ponds in nitrogen removal. Körner and Vermaat (1998) reported 73 to 97% removal of the initial Kjeldahl-nitrogen within 3 days in laboratory scale duckweed-covered systems (18.5 cm diameter and 4.5 cm depth). Alaerts *et al.* (1996) showed via mass balance calculations that 42.2% of the nitrogen removed in a full scale sewage fed lagoon was incorporated in duckweed biomass. Buddhavarapu and Hancock (1991) reported NH_4^+ removal efficiency of 70% in duckweed-based pond receiving secondary treated domestic wastewater with NH_4^+ concentration of 0.1-10 mg/l. This experiment was conducted in a pond of 1.8 m depth and a HRT of 13 days. Kawabata *et al.* (1986) reported that 70% of the nitrogen supplied to a paddy field in the form of secondary treated sewage was taken up by duckweed. It should be noted that in this case nitrogen content in the treated effluent was extremely low (4 mg-N/l). Oron *et al.* (1985) reported a total NH_4^+ removal in the range of 40-70% in semi continuous duckweed ponds. These authors used raw sewage with initial NH_4^+ concentrations of 47.5 ± 16 mg/l and COD concentrations of 318 ± 69 mg/l. These experiments were conducted in mini-ponds with water depths of 20 and 30 cm and a HRT of 3 and 10 days.

Direct comparison of results from the above studies is not possible due to differences in HRT, water depths, initial nitrogen concentrations and duckweed species, densities and harvesting regimes. The relative importance of different N-removal mechanisms and the effect of sewage composition, pond design and operation are not known and need further investigation.

DIFFERENCES BETWEEN ALGAE AND DUCKWEED-BASED PONDS

Differences in BOD removal

BOD removal mechanisms in DBP are not fully understood. It has been suggested that *Lemnaceae* can take up simple amino acids and other organic compounds from the water (Hillman, 1961; Landolt, 1986), but, Körner *et al.* (1998) concluded from laboratory studies with *Lemna gibba* that heterotrophic uptake of small organic compounds is not important. Nevertheless, they found that COD removal was significantly faster in the presence of duckweed than in uncovered controls.
Few comparative studies with respect to BOD removal have been reported in literature for wastewater treatment ponds with and without duckweed. Mandi *et al.* (1993) compared two experimental stabilisation ponds one with and another without water hyacinth in the arid climate of Morocco. They showed that total BOD, COD and TSS removal efficiencies were higher in the water hyacinth system, even at considerably shorter retention time.

Differences in TSS removal

The removal mechanisms of TSS in pond systems involve two main processes: sedimentation and biodegradation of organic particles. Effective sedimentation is important in order to increase the retention time of slowly degradable organic matter. Efficient removal of 80% of TSS has been reported in DBP systems (Mandi, 1994; Bonomo *et al.*, 1996; Zirschky and Reed, 1988). The effluents from conventional WSP systems are characterised by a high concentration of suspended solids, which in some occasions can exceed 100 mg/l due to algae in the effluent (Reed *et al.*, 1995).

Differences in pathogen removal

Pathogen removal in pond systems has been attributed to a number of natural decay processes including: (a) DNA damage caused by the UV component of solar radiation (Curtis *et al*., 1992a,b); (b) photo-oxidation (Curtis *et al*., 1992a,b); (c) bactericidal effects of algae growth (Parhad and Rao, 1974) and (d) starvation due to lack of nutrients or carbon source. These processes were found to be affected by environmental factors, such as pH, DO, temperature, solar radiation, algae biomass and nutrient concentrations (Frijns and Lexmond, 1991). Bacteria pathogens would likely be removed to somewhat lesser degree in duckweed ponds than algae ponds because of the restricted sunlight penetration and the absence of very alkaline conditions, which occur during the daytime in algae ponds. On the other hand, Alaerts *et al*. (1990) suggests that water temperature and retention time are more determining factors. Many studies have been conducted to determine the pathogen removal efficiency in WSP systems. These systems can effectively reduce pathogen counts to a level low enough for the effluent to be used for restricted irrigation (WHO, 1989).

Differences in biological and chemical nitrogen conversion and removal processes

So far, limited information is available on the fate of nitrogen in algae-based ponds and even less information is available for macrophyte systems, such as duckweed-based ponds. Below a summary is presented of existing information.

Ammonia volatilisation

Ammonia in water may be present in non-ionised (NH_3) and ionised (NH_4^+) forms. NH_3 concentration in solution rises with increases in pH and temperature. NH_3 however, is a volatile compound and therefore will be lost to the atmosphere. Because of this, the equilibrium will be continuously pulled in the direction of NH_3. The increase of pH (8-10) as a result of intense photosynthetic activity (CO_2 uptake) in WSP will stimulate ammonia volatilisation. NH_4^+ removal in WSP systems is mainly attributed to ammonia volatilisation during periods of high temperatures and pH (Pano and Middlebrooks, 1982; Silva *et al.*, 1995; Soares *et al.*, 1996). The duckweed cover prevents elevation of pH as a consequence of limited algae growth due to the shading provided by the duckweed cover. Therefore the unionised fraction of ammonia will be kept low and volatilisation is expected to be limited. In addition, the duckweed cover may provide a physical barrier for volatilisation of dissolved ammonia. Studies of duckweed covered stabilisation ponds based on incomplete mass balance equations suggested that ammonia volatilisation is not very important in overall nitrogen removal (Alaerts *et al*., 1996; Vermaat and Hanif, 1998; Körner and Vermaat, 1998).

Nitrification and denitrification

Autotrophic nitrification is achieved in two steps that are catalysed by different species of nitrifying microorganisms:

$$NH_4^+ + 1.5\ O_2 \xrightarrow{Nitrosomonas} NO_2^- + 2H^+ + H_2O \quad (1)$$

$$NO_2^- + 0.5\ O_2 \xrightarrow{Nitrobacter} NO_3^- \quad (2)$$

Both *Nitrosomonas* and *Nitrobacter* are aerobic, chemolithoautotrophs, which means that the energy from the oxidation of inorganic nitrogen compounds is used for assimilation of CO_2. Bacteria of the genus *Nitrosomonas* perform the first oxidation step to NO_2^-, whereas bacteria of the genus *Nitrobacter* perform the second oxidation step to NO_3^-. Autotrophic nitrification is amongst other factors, affected by temperature, pH and oxygen concentration. The optimum temperature is between 25 to 35^{o}C and the optimum pH is between 7 to 8 (Metcalf and Eddy, 1991). Dissolved oxygen concentration of about 2 mg/l is required for effective nitrification. Heterotrophic nitrifying bacteria can oxidise both inorganic and organic nitrogen compounds (Verstraete and Alexander, 1973; van Luijn, 1997). Laurent (1971), as cited by Green *et al.* (1996) attributed the phenomenon of heterotrophic nitrification to bacteria of the genus *Arthrobacter* in the presence of undetectable traces of O_2. Despite better growth of heterotrophs at low oxygen concentration, autotrophic nitrification appears to be more important in overall nitrification (Henriksen and Kemp, 1988). Nitrifying bacteria are known to occur attached to particles, surfaces and flocs (Focht and Verstraete, 1977; Underhill and Prosser; 1987; Verhagen and Laanbroek, 1991).
Denitrification is the dissimilatory reduction of oxidised nitrogen into gaseous N-oxides or N2 by facultative and anaerobic organisms. These bacteria are mostly heterotrophic and prefer oxygen over any other electron acceptor. In the presence of

oxygen no NO_x^- (NO_2^- or NO_3^-) is used, but in the absence of oxygen, NO_2^-/NO_3^- act as terminal electron acceptors for respiration (Knowles, 1982). Denitrification under aerobic conditions is also possible. Robertson (1988) found that *Thiosphaera pantotropha* can denitrify in the presence of both O_2 and NO_x^- in industrial wastewater. This is contrary to the commonly accepted view that dissolved oxygen will suppress the enzyme system needed in the denitrification process (Metcalf and Eddy, 1991). In general, denitrification occurs under anoxic conditions if enough organic matter is available. The presence of an energy source and an electron donor in the form of organic compounds is a prerequisite. Denitrifying bacteria are capable of dissimilatory nitrate reduction in a two step process. The first step consists of the conversion of nitrate to nitrite. This step is followed by production of nitric oxide, nitrous oxide and nitrogen gas. The sequence of conversions involved in nitrate reduction is as follows:

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Ferrara and Avci (1982) stated that ample evidence has been presented in the literature demonstrating that nitrification does not occur in stabilisation ponds under normal conditions. Most authors agreed to this assumption because low concentrations of nitrite and nitrate were found in the effluent. However, low concentrations of both nitrite and nitrate do not prove that nitrification does not occur in ponds and in fact may be due to efficient denitrification. In some studies in macrophyte systems, denitrification was referred to as unaccounted for nitrogen and found to contribute for 10-20% to the nitrogen loss (Alaerts *et al.*, 1996; Körner and Vermaat, 1998; Vermaat and Hanif, 1998).

Nitrogen fixation

Zuberer (1982) and Duong and Tiedje (1985) demonstrated nitrogen fixation associated with duckweed covers. In this process atmospheric nitrogen is reduced to NH_4^+ which is subsequently incorporated in biomass. Nitrogen fixation usually does not occur if ammonia is present in the environment. In the presence of ammonia nitrogenase synthesis is suppressed by a phenomenon called the "ammonia switch-off" effect (Brock *et al.*, 1991). Duong and Tiedge (1985) reported a N-input in naturally occurring duckweed-cyanobacterial associations of 1-2 mg-N $m^{-2}d^{-1}$. This value is low compared to the total amount of nitrogen fed to the treatment ponds via the wastewater (possibly 1-2 g-N $m^{-2}d^{-1}$). N-fixation therefore is not likely to affect the overall nitrogen balance in wastewater treatment ponds.

SCOPE OF THIS THESIS

This thesis presents the results of research on a natural treatment technology, which is suitable for nutrient recovery and effluent reuse. So far few studies have been undertaken to compare the general performance and N-transformation mechanisms of ABPs and DBPs systems. Nitrogen in wastewater is an important pollutant causing eutrophication in surface water and causes ground water pollution by nitrate. In recognition of the deteriorating effects of nitrogen on water resources, standards for effluent discharges are becoming stricter, and nutrient removal becomes one of the prerequisites for selection of wastewater treatment systems. Therefore, in this study, individual nitrogen transformation processes and removal mechanisms were assessed and nitrogen mass balances were made based on all nitrogen fluxes.

This thesis starts with introduction (this chapter) and concludes with a summary. The contents of the remaining chapters describe the results of a research concerning the comparison of algae and duckweed-based systems for wastewater treatment with emphasis on nitrogen fluxes and removal mechanisms. Time schedule indicating the duration of the various experiments carried out in the scope of this thesis and the type of wastewater used is shown in Table 2. In chapter 2 in order to understand the nitrogen removal by different mechanisms in algae and duckweed-based waste stabilisation ponds, outdoor batch incubations of wastewater were conducted. Different N-removal mechanisms under different conditions of DO and pH were investigated. The various mechanisms were subsequently investigated in continuous flow systems consisting of four ponds in series for each treatment type. In Chapter 3 a tracer experiment is described which was carried out in the pilot-scale algae and duckweed-based system at Birzeit University, Palestine, to study the differences in hydraulic behaviour between the two systems.

Larger scale algae pond and duckweed pond were studied in Ginebra, Colombia and the results reported in the same chapter. Chapter 4 compares seasonal performance and removal efficiencies of the two systems at Birzeit when fed with low strength wastewater in terms of organic content. Removal of BOD, TSS, nutrients and faecal coliforms were investigated. Chapter 5 compares the performance and removal efficiencies of the two systems at Birzeit when fed with wastewater of low and high strength. Parameters compared were as in the previous chapter. In chapter 6 a comparison of ammonia volatilisation rates in algae and duckweed-based ponds is presented. This chapter also describes the development and application of a simple method to assess the relative contribution of ammonia volatilisation to overall N-removal from domestic waste stabilisation ponds. It also discusses models from literature derived to predict ammonia volatilisation in stabilisation ponds. Chapter 7 deals with measurements of nitrification and denitrification rates at the top, in the middle and just above the sediment in the series of the four algae and duckweed-based ponds in laboratory batch incubations. Effect of temperature and BOD loading was investigated. In situ measurements over the pond depth were also done for confirmation of laboratory results. Finally, in Chapter 8 nitrogen mass balances are presented, based on measured nitrogen fluxes in both algae and duckweed-based ponds. Comparisons were made during different periods characterised by different seasonal effects and wastewater composition. The relative importance of various nitrogen fluxes and removal pathways was quantified and discussed.

Table 2. Time schedule indicating the duration of the various experiments carried out in the scope of this thesis and the type of wastewater used.

Dec-98	Feb-99	Apr-99	Jun-99	Aug-99	Oct-99	Dec-99	Feb-00	Apr-00	Jun-00	Aug-00	Oct-00	Dec-00	Feb-01	Apr-01	Jun-01	Aug-01
Source of Wastewater																
WW of Low organic strength											WW of high N		WW of high organic strength			
→	Start of monitoring scheme															
Chapter 2														Warm season		
Chapter 3							Cold season				Ginebra-Colombia					
Chapter 4	Seasonal variations															
Chapter 5									Warm season						Warm season	
Chapter 6						Cold and warm season										
Chapter 7						Cold season		Warm season							Warm season	
Chapter 8						Cold season		Warm season							Warm season	

REFERENCES

Alaerts G.J, Veenstra S., Bentvelson M. and van Duijl L.A. (1990). Feasibility of anaerobic sewage treatment in sanitation strategies in developing countries. IHE, Report Series 20, Delft.

Alaerts G.J., Mahbubar M.R. and Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res*. **30:** 843-852.

Al-Nozaily, F. (2001). Performance and process analysis of duckweed-covered sewage lagoons for high strength sewage. The case of Sana'a Yemen. Ph.D. Thesis, IHE, The Netherlands.

Araujo M.C. (1987). Use of water hyacinth in tertiary treatment of domestic sewage. *Wat. Sci. Tech*. **19:**11-17

Bergen V.W.J.v.d. (1997). Challenges in urban water management. Proceeding of the Third Japan-Netherlands Workshop on Integrated Water Quality Management.

Bonomo L., Pastorelli G. and Zambon N. (1996). Advantages and limitations of duckweed-based wastewater treatment systems. *5th International Conference on Wetland System for Water Pollution Control*, VIII/2, 1-8, Vienna.

Brix H. and Schierup H. H. (1989). The use of aquatic macrophytes in water pollution control. *Ambio*. **18:** 100-107.

Brock T.D., Madigan M.T., Martinko J.M. and Barker J. (1991). Biology of microorganisms. 17th edition, Prentice Hall Inc., London.

Bucks D. A., Nakayama F.S. and Gilbert R.G. (1979). Trickle water quality and preventive maintenance. *Agricultural Water Management*. **2:** 149-162.

Buddhavarapu L.R. and Hancock S.J. (1991). Advanced treatment for lagoons using duckweed. *Wat. Environ. Technol*. **3:** 41-44.

Corradi M., Copelli M. and Ghetti P. F. (1981). Olture di Lemna su scarichi zootechnici. Inquinamento. **23:** 49-45.

Cosgrove W.J. and Rijsberman F.R. (2000). World Water Vision, Making Water Everybody's Business. The World Water Council, Earthscan Publ. Ltd., UK, pp 108

Culley D.D., Gholson J.H., Chisholm T.S., Standifer L.C. and Epps E.A. (1978). Water quality renovation of animal waste lagoons utilising aquatic plants. U.S. EPA, Ada, Oklahoma. pp. 166.

Curtis T. P., Mara D. D. and Silva S. A. (1992a). "Influence of pH, oxygen and humic substances on ability of solar radiation to damage FC in waste stabilisation pond water." *Appl. Envir. Microbilol*. **58**(4): 1335-1343.

Curtis T. P., Mara D. D. and Silva S. A. (1992b). "The effect of sunlight on faecal coliforms in ponds, implications for research and design." *Wat. Sci. Tech*. **26:** 1729-1738.

Duong T.P. and Tiedje J.M. (1985). Nitrogen fixation by naturally occurring duckweed-cyanobacterial associations. *Canadian Journal of Microbiology*. **31:** 327-330.

Edwards P. (1980). Food potential of aquatic macrophytes. *ICLARM Studies and Reviews* **5:** 51 pp.

Ferrara R. A. and Avci C. B. (1982). Nitrogen dynamics in waste stabilisation ponds. *J. WPCF*. **54**(4): 361-369.

Focht D.D. and Verstraete W. (1977). Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology*. **1:** 135-198.

Frijns J.A.G. and Lexmond M.J. (1991). Removal of pathogenic organisms by biological wastewater treatment. Internal report Nr. 91-6. Department of Environmental Technology, Wageningen Agricultural University, The Netherlands.

Gijzen H.J. (1999). Sustainable wastewater management via re-use: *Turning waste into wealth.* In: Garcia M. *et al* (eds.) Proc. AGUA98 –Water and Sustainability, Cali, June 1-3, 1998, pp 211-224.

Gijzen H.J. (1996). Anaerobic wastewater treatment: an important step in rational reuse strategies of nutrients and energy. In: Proc. IV Seminario-Taller Latino Americano.

Gijzen H.J. (2001a). Aerobes, anaerobes and phototrophs: a winning team for wastewater manangement. *Wat.Sci.Tech*. **44**(8): 123-132.

Gijzen H.J. (2001b). Low cost wastewater treatment and potentials for re-use: a cleaner production approach to wastewater management. In: proceedings Intl. Symposium on low cost wastewater treatment and re-use. Cairo, Egypt, February 3-4.

Gijzen H.J. (2002). Anaerobic digestion for sustainable development: a natural approach. *Wat. Sci. Tech*. **45**(10):321-328.

Gijzen H.J. and Ikramullah M. (1999). Pre-feasibility of duckweed-based wastewater treatment and resources recovery in Bangladesh. World Bank Report, Washington D.C., 194.

Gijzen H.J., Siebel M. and Nhapi I. (in prep.). A strategic approach to wastewater management.

Grau P. (1994). What's next? *Wat. Qual. Intl*. **4:** 29-32.

Green F.B., Bernstone L.S., Lundquist T.J. and Oswald W.J. (1996). Advanced integrated wastewater pond systems for nitrogen removal. *Wat. Sci. Tech*. **33:** 207-217.

Gunnerson C.G. and French J.A. (1996). Wastewater management for coastal cities – The ocean disposal option. Sringer Verlag, Berlin. pp 340.

Hammes F., Kalogo Y. and Verstraete W. (2000). Anaerobic digestion technologies for closing the domestic water, carbon and nutient cycles. *Wat. Sci. Tech*. **41:** 203-211.

Harremoës P. (2000). Advanced water treatment as a tool in water scarcity management. *Wat.Sci.Tech*. **42**(12): 73-92.

Henriksen K. and Kemp W.M. (1988). Nitrification in estuarine and coastal marine sediments. In: Nitrogen cycling in coastal marine environments. Y.H. Blackburn and J. SØrensen [eds.], John Wiley and Sons Ltd., New York.

Hillman S. (1961). The *Lemnaceae*, or duckweed, a review of descriptive and experimental literature. *Botanical Reviews*. **27:** 221-287.

ICDDRB International Ceter for Diarrhoeal Diseases Research, Bangladesh (1995). Unpublished data. ICDDRB, Ghaka.

Kawabata Z., Tatsukawa R. and Sato K. (1986). Growth of duckweed and nutrient removal in a paddy field irrigated with sewage effluent. *Journal Environmental Studies*. **27:** 277-285.

Knowles R. (1982). Denitrification. *Microbiological Reviews*. **46:** 43-70.

Körner S. and Vermaat J. E. (1998). The relative importance of *Lemna gibba* L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Wat. Res*. **32**(12): 3651-3661.

Körner S., Lyatuu G. B. and Vermaat J. E. (1998). The influence of *Lemna gibba* L. on the degradation of organic material in duckweed-covered domestic wastewater. *Wat. Res*. **32**(10): 3092-3098.

Kvet J., Rejmankova E. and Rejmanek M. (1979). High aquatic plants and biological waste water treatment. The outline of possibilities (In Czech). *Proceedings of the Aktiv Jihoceskych Vodohospodaru Conference*. pp 9.

Landolt E. (1986). The family of *Lemnaceae* - a monographic study. *Veröffentlichungen des Geobotanischen Institutes der ETH Zürich*, Band **71:** 566 pp.

Larsen T.A. and Guyer W. (1996). Separate management of anthropogenic nutrient solutions (human urine). *Wat. Sci. Tech*. **34**(3-4): 87-94.

Lettinga G., Lens P. and Zeeman G. (2001). The DESAR concept for environmental protection. In: Decetralised sanitation and re-use; Concepts, systems and implementation. Eds. Piet Lens, Grietje Zeeman & Gatze Lettinga. IWA publishing.

Lettinga G., van Velsen A.F.M., Hobma S.W., de Zeeuw W. and Klapwijk A. (1980). Use of the upflow sludge balanket (UASB) rector concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng*. **22:** 699-734.

Lüönd A. (l980). Effects of nitrogen and phosphorus upon the growth of some *Lemnaceae*. In: *Biosystematische Untersuchungen in der Familie der Wasserlinsen (Lemnaceae), Veröffentlichungen des Geobotanischen Institutes der ETH Zürich, Landolt E.* Band **70:** 118-141.

Mahmoud N. (2002). Anaerobic pre-treatment of sewage under low temperature (15^oC) conditions in an integrated UASB-digester system. PhD. Thesis, Wageningen University, The Netherlands.

Mandi L. (1994). Marrakesh wastewater purification experiment using vascular aquatic plants *Eichhornia crassipes* and *Lemna gibba. Wat. Sci. Tech.* **29:** 283-287.

Mandi L., Ouazzani N., Bouhoum K. and Boussaid A. (1993). Wastewater treatment by stabilisation ponds with and without macrophytes under arid climate. *Wat. Sci.Tech.* **28:** 177-181.

Metcalf and Eddy (1991). Wastewater engineering. Treatment, disposal and reuse. Tchobanoglous G. and Burton F. L. [eds.], McGraw – Hill. New York.

Mujeriego R. and Asano T. (1991). Wastewater reclamation and reuse. Pergamon Press, Oxford.

Murray W.D. and van der Berg L. (1981). Effect of support material on development of microbial fixed films converting acetic to methane. *J. Appl. Bacteriol*. **51:** 257-265.

Niemczynowics J. (1997). The water profession and agenda 21. *Wat. Qual. Int*. **2:** 9-11.

Oron G. (1990). Economic considerations in wastewater treatment with duckweed for effluent and nitrogen renovation. *J. WPCF*. **5:** 692-696.

Oron G. (1994). Duckweed culture for wastewater renovation and biomass production. *Agric. Water Man*. **26:** 27-40

Oron G., Wildschut L.R. and Porath D. (1985). Waste water recycling by duckweed for protein production and effluent renovation. *Wat. Sci. Tech.***17**: 803-817.

Pano A. and Middlebrooks E.J. (1982). Ammonia nitrogen removal in facultative wastewater stabilisation ponds. *J. WPCF*. **54:** 344-351.

Parhad N.M. and Rao N.U. (1974). Effect of pH on survival of *Escherichia coli. J. WPCF*. **46**(5): 980-986.

Pearson H.W., Mara D.D. and Arridge H.A. (1995). The influence of pond geometry and configuration on facultative and maturation waste stabilisation pond performance and efficiency. *Wat. Sci. Tech.* **31**(12): 129-139.

Porath D. and Pollock J. (1982). Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquatic Botany*, **13:** 125-131.

Reddy K. R. and DeBusk T.A. (1987). State of the art utilisation of aquatic plants in water pollution control. *Wat. Sci. Tech.* **19**(10): 61-79.

Reddy K. R. and DeBusk W. F. (1985). Nutrient removal potential of selected aquatic macrophytes. *Journal of Environmental Quality*. **14:** 459-462.

Reed S.C., Crites R.W. and Middlebrooks E.J. (1995). Natural systems for management and treatment. McGraw - Hill. New York.

Robertson L. A. (1988). Aerobic denitrification and heterotrophic nitrification in *Thiosphaera pantotropha* and other bacteria. Ph.D. Thesis. Delft Technical University, Delft, The Netherlands.

Shuval H.I., Adin A., Fattal B., Rawitz E. and Yekutiel P. (1986). Wastewater irrigation in developing countries: Health effects and technical solutions. The World Bank, Washington DC. World Bank Technical Paper. **51:** 324 pp.

Silva S.A., de Oliveira R., Soares J., Mara D.D. and Pearson H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Wat. Sci. Tech.* **31**(12): 321-330.

Skillicorn P., Spira W. and Jouney W. (1993). Duckweed Aquaculture, a new aquatic farming system for developing countries. The World Bank, Washington, USA.

Soares J., Silva S.A., de Oliveira R., Araujo A.L.C., Mara D.D. and Pearson H.W. (1996). Ammonia removal in a pilot-scale WSP complex in Northeast Brazil. *Wat. Sci. Tech.* **33:** 165-171.

Sutton D.L. and Ornes W.H. (1977). Growth of *Spirodela polyrrhiza* in static sewage effluent. *Aquatic Botany*. **3:** 231-237.

Switzenbaum M.S. and Jewell W.J. (1980). Anaerobic attached film extended-bed reactor treatment. *J. WPCF*. **52:** 1953-1965.

Tripathi B. D., Srivastava J. and Misra K. (1991). Nitrogen and phosphorus removal capacity of four chosen aquatic macrophytes in tropical freshwater ponds. *J. Env. Qual*. **18:** 143-147.

Underhill S.G. and Prosser J.I. (1987). Surface attachment of nitrifying bacteria and their inhibition by potassium ethylxanthate. *Microbial Ecology*. **14:** 129-139.

UNEP/GPA (2000). Strategy options for sewage management to protect the marine environment. Report produced by IHE-Delft for UNEP/GPA, November 2000. pp102.

van der Steen P., Brenner A. and Oron G. (1998). An integrated duckweed and algae pond system for nitrogen removal and renovation. *Wat. Sci. Tech*. **38:** 335-343.

van Luijn F. (1997). Nitrogen removal by denitrification in the sediments of a shallow lake. Ph.D. Thesis. University of Wageningen, Wageningen, The Netherlands.

Verhagen F.J.M. and Laanbroek H.J. (1991). Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. *Appl. Environ. Microbiol*. **57:** 3255-3263.

Vermaat J. E. and Hanif M.K. (1998). Performance of common duckweed species (*Lemnaceae*) and the *Waterfern Azolla Filiculoides* on different types of wastewater. *Wat. Res*. **32:** 2569-2576.

Verstraete W. and Alexander M. (1973). Heterotrophic nitrification in samples of natural ecosystems, *Environmental Science and Technology*. **7:** 39-42.

WHO (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. *Technical report series 778*. World Health Organisation, Geneva, Switzerland.

WHO (1996). Water supply and sanitation sector monitoring. *Report 1996*: "Sector status as of 31 December 1994". In WHO/EOS/96.15. Geneva, Switzerland.

World Bank (1982). Appropriate Sanitation Alternatives: Volume I and II.

Young J.C. and McCarty P.L. (1967). The anaerobic filter for wastewater treatment. In: Proc. 22nd Purdue Industrial Waste Conference, May 1967. Ann Arbor Science Publishing Inc., Ann Arbor MI. 559-574.

Zeeman G. and Lettinga G. (1999). The role of anaerobic digestion in closing the water and nutrient cycle at community level. *Wat. Sci. Tech*. **39**(5): 187-194.

Zirschky J. and Reed S. C. (1988). The use of duckweed for wastewater treatment. *J. WPCF*. **60:** 1253-1258.

Zuberer D.A. (1982). Nitrogen fixation (acetylene reduction) associated with duckweed (*Lemnaceae*) mats. *Appl. Environ. Microbiol*. **43:** 823-828.

Chapter 2

EFFECT OF DISSOLVED OXYGEN AND pH ON NITROGEN REMOVAL IN BATCH INCUBATIONS SIMULATING ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

Submitted as:

Zimmo O. R., Al-Sa'ed R. M., Van der Steen N. P. and Gijzen H. J. Effect of dissolved oxygen and pH on nitrogen removal in batch incubations simulating algae and duckweed-based waste stabilisation ponds. *Wat. Res.*

EFFECT OF DISSOLVED OXYGEN AND pH ON NITROGEN REMOVAL IN BATCH INCUBATIONS SIMULATING ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

ABSTRACT

Twelve incubations of algae and duckweed mini-ponds, containing domestic wastewater were monitored during 15 day batch trials to study the contributions of different N-removal mechanisms under different conditions of DO and pH. N-removal by different mechanisms in both systems appeared more dependent on the pH variations than on oxygen variations. At high pH range (7-9), N-removal efficiencies were significantly higher compared to low pH range (5-7) in both systems. Irrespective of oxygen condition, significantly higher nitrogen removal efficiencies occurred in duckweed system (26-33%) than algae system (14%-24%) at the lower pH range of 5-7. The principal N-removal mechanisms in duckweed system at low pH were N-uptake by duckweed and N-removal via uptake by biomass attached to container wall and in the sediment. At high pH values (7-9), the increase in N-removal by sedimentation in algae system and the decrease in N-uptake by duckweed in duckweed system resulted in significantly higher N-removal in the algae incubation (45-60%) compared to the duckweed incubation (38-41%). N-removal in the algae incubation without oxygen control at high pH range (7-9) was 17-20% higher compared to the anaerobic and aerated incubations, due to higher nitrification/denitrification and sedimentation. In duckweed systems this was 22-25% higher.

Keywords: Algae, dissolved oxygen, duckweed, nitrogen mechanisms, pH, stabilisation ponds

INTRODUCTION

Sustainable technologies for domestic wastewater treatment should be aimed at effective recovery of energy and nutrients from domestic wastewater (Gijzen, 2001). Both conventional algae-based waste stabilisation ponds (ABPs) and duckweed-based ponds (DBPs) enable nitrogen reuse, either through effluent irrigation or through animal feed production. DBP systems might have several advantages over ABP systems: algal growth is prevented by the presence of a duckweed cover, while nitrogen can be effectively removed by regular harvesting of the duckweed biomass. The algae in ABPs trigger diurnal differences in dissolved oxygen (DO) and pH, while DBPs usually maintain rather stable conditions (Zimmo *et al*., 2000). DO and pH are important parameters in both chemical and biological nitrogen transformation processes such as ammonia volatilisation, nitrification and denitrification and incorporation in micro and macrophyte biomass. DO is an important factor for nitrification, the limiting step in overall nitrification/denitrification. In ABP, DO is high due to algal photosynthesis activity. In DBPs re-aeration through the surface might be obstructed by the duckweed cover (O'Brien, 1981; Zirschky and Reed, 1985), resulting in lower DO concentration than in ABPs. Higher pH shifts the equilibrium of ammonia towards more unionized ammonia and increases the potential of ammonia volatilisation. In previous studies in waste stabilisation ponds, models were developed to predict nitrogen removal incorporating pH as an important factor (Reed, 1985; Silva *et al*., 1995) with the assumption that ammonia volatilisation is the principal

mechanism for removal. High pH values are not likely to occur in DBPs, but if it occurs, it has a detrimental effect on duckweed (Wang, 1991; Clement and Merlin, 1995; Caicedo *et al*., 2000). In that case nitrogen removal via duckweed harvesting would be reduced. Optimum range of pH found in literature for the growth of duckweed are within the range between 5 to 8 (Mclay, 1976; Porath and Pollock, 1982; Zirschky and Reed, 1988; Skillicorn *et al*., 1993). Because of the neutral value of pH in DBPs, it was assumed that ammonia volatilisation was not likely to occur (Alaerts *et al*., 1996; Körner and Vermaat, 1998; Zimmo *et al*., 2000) and the loss of nitrogen that could not be accounted for were attributed mainly to denitrification.
Research works comparing nitrogen transformations and removal in algae and duckweed-based wastewater systems at different pH and DO concentration is lacking. This is the subject of the present study.

MATERIALS AND METHODS

Experimental set-up

Outdoor incubations were carried out in the vicinity of the wastewater treatment plant at Birzeit University-Palestine. The experiments consisted of batch incubations of domestic wastewater in algae-based and duckweed (*Lemna gibba*) based containers. The incubations were conducted to study the effect of dissolved oxygen (DO) and pH on mechanisms of nitrogen removal. Each incubation was conducted in triplicate to compare duckweed-based systems with algae-based systems. Incubations 1 to 4 were conducted under anaerobic condition with the pH as experimental variable. The pH in the four incubations was kept within the following ranges: 5-6, 6-7, 7-8 and 8-9, respectively. Incubations 5-8 (controls) were conducted using the same pH ranges but under natural (uncontrolled) variation of DO. Incubations 9-12 were conducted using the same pH ranges but under fully aerated condition. Each incubation was done with and without the addition of 240 mg of nitrification inhibitor (1-Allyl-2-thiourea (ATU), MERCK) to the 12 liter containers (20 mg l^{-1}). In this way the N-removal without the influence of nitrifiers was quantified.
Settled wastewater from Birzeit University was used to start incubations. Nitrogen content was adjusted to 100 mg-N l^{-1} by adding NH_4Cl. Physico-chemical characteristics of wastewater used in the incubations are shown in Table 1.

Table 1: Physico-chemical characteristics of wastewater used in the batch incubations. Values are averages (±SD). All values are in mg l^{-1} unless otherwise stated.

Parameter	Concentration
Total nitrogen	106 (3.2)
NO_3^--N	0.1 (0.1)
COD	201 (10)
Total-P	4.5 (0.3)
pH (-)	7.8 (0.2)
DO	0.2 (0.1)
Temperature (^oC)	25.5 (2.2)

Plastic containers of 12 liter volume, 28.5 cm diameter and 30 cm depth were used for incubation. Duckweed-based systems were stocked with 35.6 g fresh weight of *L.*

gibba which resulted in a density of 600 g fresh weight m^{-2}. Algae containers were seeded with algae to a final concentration of 10 mg/L. The algae were collected by centrifugation of samples from pilot-scale stabilisation ponds at Birzeit University. The increase in initial nitrogen and phosphorus content of the container by the introduction of algae was calculated to be negligible (maximum 1% and 3% based on the assumption that the nitrogen and phosphorus contents of algae are 5% and 1% of the dry weight, respectively). This represents approximately 0.5% of the initial amount of nitrogen present in the container. Anaerobic conditions during incubations 1-4 were maintained by adding Na_2SO_3. For the aerated incubations (Inc. 9-12), the containers were continuously aerated by an aquarium air pump. The pumping rate was 0.7 l/s and the air bubbles were trapped in inverted small cups to keep the water surface undisturbed. pH in the containers was monitored twice a day to maintain the designated values. Diluted NaOH or HCl was used to adjust the pH, whenever necessary. Water loss by evaporation from algae-based and evapotranspiration from duckweed-based containers was compensated for by addition of demineralised water (approximately 600 ml per container every 2 days). Each incubation was monitored for the 15 days incubation period. The contribution of the different mechanisms of nitrogen removal in algae and duckweed-based systems was calculated as follows:

$$(N_i - N_f) = N_s + N_{dw} \text{ (for duckweed-based system only)} + N_{AV} + N_{deni} \qquad (1)$$

$(N_i - N_f)$ is the initial total nitrogen minus the final total nitrogen content of wastewater in the 12 liter containers, which represents the N-removal via biofilm growth (algae and bacteria) on the walls and by sedimentation (N_s), the N-uptake by duckweed (N_{dw}), N-removal via ammonia volatilisation (N_{AV}) and N-removal via denitrification (N_{deni}). N-removal via ammonia volatilisation is calculated as the difference between $(N_i - N_f)$ and the sum of N_s and N_{dw} in incubations with nitrification inhibitor. The difference between $(N_i - N_f)$ and the sum of N_s and N_{dw} in incubations without nitrification inhibitor represents the N-removal via ammonia volatilisation and denitrification together. N-removal via denitrification is therefore estimated as the difference between ammonia volatilisation and denitrification together (incubations without nitrification inhibitor) and ammonia volatilisation alone (incubations without nitrification inhibitor). For the algae-based system the same mass balance equation was used but without the component of N_{dw}.

Sampling and analysis

Twice a day DO was measured using DO175 (HACH), temperature and pH values were measured using EC10 pH meter (HACH). DO and pH were adjusted to the set value. Water samples were taken every five days at 9:00 a.m. from mid-depth of each container since analysis showed negligible variations in concentration with depth. Kjeldahl-nitrogen (N_{kj}) was analysed according to Standard Methods (APHA, 1992). NO_x^--N: Nitrite (NO_2^--N) and Nitrate (NO_3^--N) were analysed according to the Advanced Water Quality Laboratory Procedures Manual by HACH. Dry weight (after drying at 70 ^oC for two days) of duckweed was measured at the beginning of the incubation (DW_o) (using typical representative samples) and after harvesting (DW_1). Samples from dried duckweed were analysed for nitrogen tissue contents using a titrimetric method (APHA, 1992) after peroxide digestion according to Novozamsky *et al*. (1983). Relative growth rates (RGR) were calculated using the following equation: RGR= $(\ln DW_1 - \ln DW_0)/t$ (Hunt, 1978) with t indicating the growth period in days. Nitrogen content in sediments was measured after collection at the end of the incubation and drying at 70 ^{o}C for two days using titrimetric method after digestion as described for duckweed. Removal coefficients for total nitrogen (Kj-N + NO_x^--N) was fitted using the exponential equation $y = z \times e^{(-k * t)}$ where z is the intercept, k is the removal coefficient day^{-1} and t is the time in days.

RESULTS

In anaerobic incubations (Inc. 1-4) the DO concentration was ranging from zero at the beginning of the incubation to less than 1 mg l^{-1} just before readjustment (every third day). In the control incubations (Inc. 5-9) DO concentrations ranged between 6 (morning) to 8 (late afternoon) and 3 to 5 mg l^{-1} in algae and duckweed systems, respectively. In aerated incubations (Inc. 9-12) DO was stable and ranged between 8 and 9 mg l^{-1}.

The presence or absence of DO was associated with the presence of NO_3^-. NO_2^- concentrations were negligible (less than 0.2 mg-N l^{-1}) in all incubations. NO_3^- concentrations ranged between 0-0.3, 6.3-15.5 and 1.4-3.1 mg-N l^{-1} in incubations 1-4, 5-8 and 9-12, respectively. Nitrogen removal was found to be more affected by variations in pH than by variations in DO (Table 2). Average initial nitrogen surface loading in both algae and duckweed systems was 21 g-N m^{-2}.

In all incubations, nitrogen removal followed an exponential reduction curve with high correlation coefficients ≥0.95. In the algae incubations, results showed that for the same pH, the values for N-removal in anaerobic and aerobic environments were not significantly different. Duckweed systems behaved likewise. However, the pH did affect N-removal since in both systems N-removal at pH range 5-7 was significantly different from the removal at pH range 7-9. At high pH range (7-9) N-removal was significantly higher in comparison with low pH ranges (5-7) in both systems.

Significantly higher overall N-removal in duckweed-based systems (26-33%; 362-454 mg-N m^2d^{-1}) than algae systems (14%-24%; 195-333 mg-N m^2d^{-1}) was found at low pH range of 5-7. However, at high pH values (7-9) the N-removal in algae systems (45-67%; 556-779 mg-N m^2d^{-1}) was significantly higher compared to duckweed systems (38-55%; 549-587 mg-N m^2d^{-1}). The nitrogen removal in the control incubations for algae at pH 7-9 was 17-20% higher than in the anaerobic and aerobic incubations at the same pH. In duckweed systems the increase was 22-25%. Good correlation between N-removal coefficients (k) and mid range pH values for the three DO levels in algae systems and duckweed systems was found (Figure 1) (R^2 ≥95). With the equations shown in Figure 1, N-removal coefficients could be calculated for the prevailing pH value and DO levels.

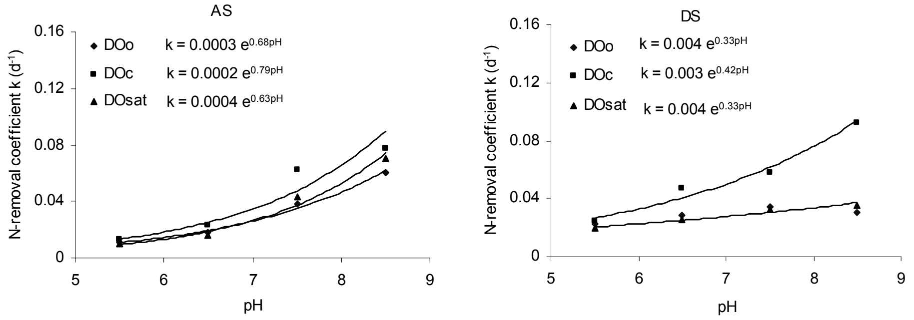


Figure 1. Nitrogen removal coefficients (k) observed in algae-based systems (ABC) and duckweed-based systems (DBC) at different pH ranges: 5-6, 6-7, 7-8 and 8-9.

The mechanisms of N-removal in algae and duckweed-based systems at different DO levels and pH ranges are shown in Figure 2 and 3. The removal was quantified either by direct measurements or by using equation 1 (mass balance equation).
N-removal by biofilm growth (algae and bacteria) on the walls and by sedimentation (N_s) in both algae and duckweed systems increased with increase in pH, and was higher in algae systems than in duckweed systems. Irrespective of DO levels, N_s in algae systems and duckweed systems at pH of 5-7 was between 9-16% and between 6-13% of initial total nitrogen concentration, respectively. Comparing N_s in algae systems for pH range of 5-7 and 7-9 shows an average increase by 45-65% at higher pH. Similarly duckweed systems shows an increase of 29-46% at higher pH.
Systematic correlation between ammonia volatilisation rates and pH values was found in algae and duckweed-based systems. Irrespective of DO level, N-removal via the ammonia volatilisation in algae systems and duckweed systems at low pH range (5-7) were not significantly different at 4-7% (68-100 mg-N $m^{-2}d^{-1}$) and 4-6% (51-87 mg-N $m^{-2}d^{-1}$) of total N input, respectively. At pH range (7-9) the removal had substantially increased to 14-28% (190-373 mg-N $m^{-2}d^{-1}$) and 9-19% (128-253 mg-N $m^{-2}d^{-1}$), respectively.
In anaerobic incubations (Inc. 1-4), nitrification was inhibited due to low DO and consequently denitrification could not occur. In aerated incubations, denitrification was inhibited due to absence of anaerobic conditions. This resulted in N-removal due to denitrification in algae systems and duckweed systems to be less than 1.2 % (less than 17 mg-N $m^{-2}d^{-1}$) and less than 4.5% (less than 58 mg-N $m^{-2}d^{-1}$) of the initial concentration, respectively. Higher denitrification was found in the control incubations since simultaneous nitrification and denitrification could occur. N-removal in the control incubations due to denitrification were 3-7% (38-97 mg-N $m^{-2}d^{-1}$) in algae system and 2%-7% (24-94 mg-N $m^{-2}d^{-1}$) in duckweed-based system at pH 5-7 and increased significantly to 19-22% (258-297 mg-N $m^{-2}d^{-1}$) and 17-19% (228-256 mg-N $m^{-2}d^{-1}$) at pH 7-9.
In all incubations of duckweed systems, N-contents of duckweed were 40.7-56.3 mg-N per g dry weight (dw). In the incubations of pH range of 5-8, RGR were not significantly different irrespective of DO and ranged between 0.04 and 0.05 day^{-1}. Increase in pH to 8-9 resulted in a decrease of RGR to -0.02 day^{-1}. In all incubations at pH range of 5-8, N-uptake rates by duckweed were ranging between 128 to 171 mg-N $m^{-2}d^{-1}$, i.e. 9 to 12% of total nitrogen input (Figure 3).

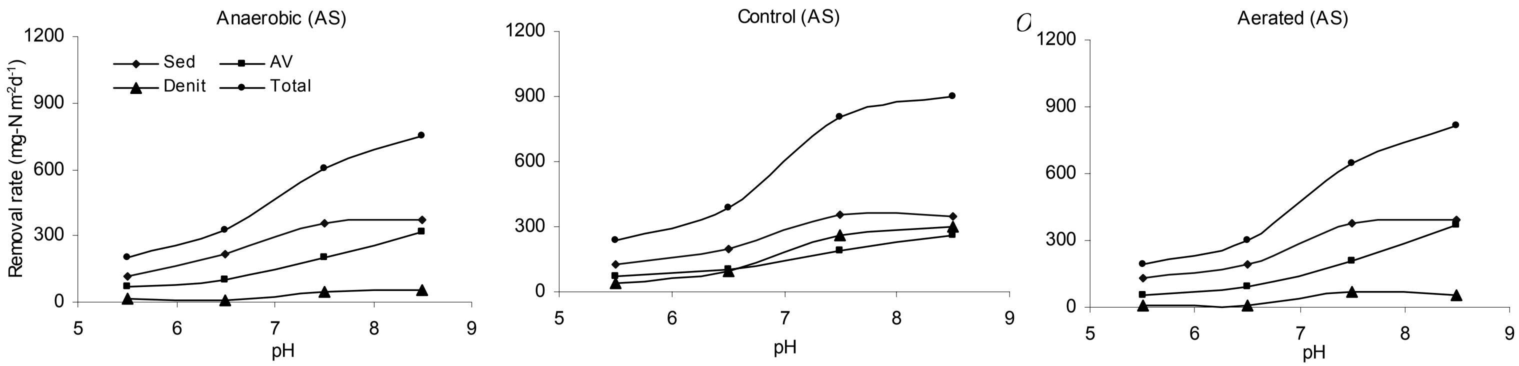


Figure 2. Nitrogen removal rates by different mechanisms in algae-based containers at different DO levels and pH ranges. Values are means of triplicates plotted at mid points of the pH ranges (5-6, 6-7, 7-8 and 8-9).

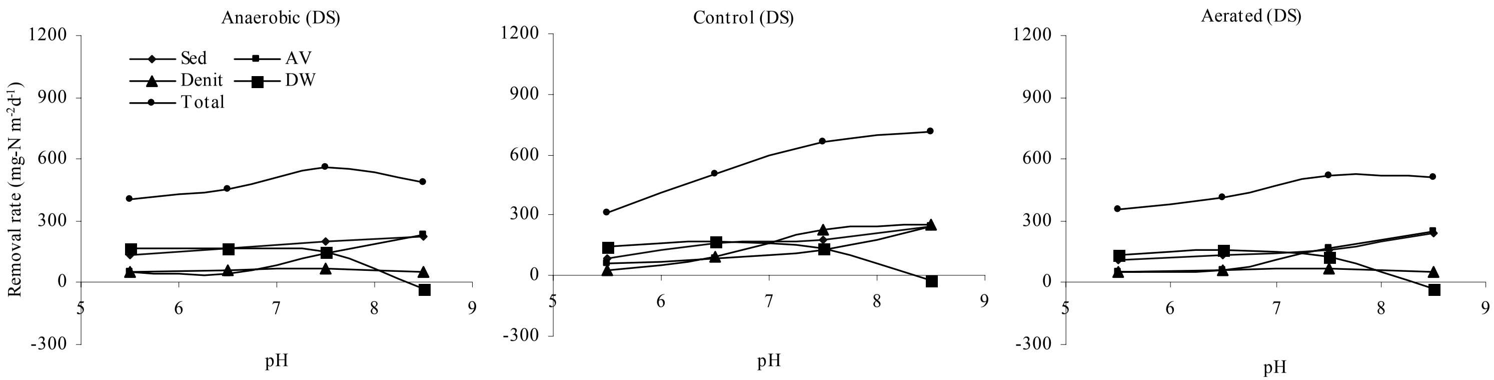


Figure 3. Nitrogen removal rates for different mechanisms in duckweed-based containers at different DO levels and pH ranges. Values are means of triplicates plotted at the mid points of the pH ranges (5-6, 6-7, 7-8 and 8-9).

Table 2. Comparison between fitted exponential nitrogen removal curves ($y = z\ e^{-kt}$), initial and final concentrations, removal efficiencies and significant differences (Sig.) in algae systems (AS) and duckweed systems (DS) for the twelve incubations (Inc.) conducted.

		Algae incubation						Duckweed incubation						AS & DS
Inc.	pH	z	k	r^2	Initial±SD (mg l-1)	Final±SD (mg l-1)	Removal (%)	z	k	r^2	Initial±SD (mg l-1)	Final±SD (mg l-1)	Removal (%)	Sig.
1	5-6	109	0.01	0.94	110±2	93±0.5	15	109	0.02	0.99	110±0.6	77±2.0	29	Y
2	6-7	108	0.02	0.98	108±0.6	82±1.8	24	109	0.03	0.98	108±2.0	72±1.0	33	Y
3	7-8	90	0.04	0.99	92±2.7	51±0.5	45	109	0.03	0.95	108±2.3	64±1.9	41	Y
4	8-9	93	0.06	0.98	90±2.8	40±1.3	56	109	0.03	0.97	107±1.5	66±1.4	38	Y
5	5-6	100	0.01	0.98	101±1.3	84±1.1	18	105	0.02	0.95	101±0.4	78±0.9	23	Y
6	6-7	102	0.02	0.99	101±1.7	72±1.3	29	108	0.05	0.95	101±1.1	63±0.5	37	Y
7	7-8	102	0.06	0.98	101±2.1	41±1.2	60	111	0.06	0.95	106±1.3	54±1.3	49	Y
8	8-9	108	0.08	0.98	105±1.5	35±0.9	67	111	0.09	0.97	101±2.0	46±1.1	55	Y
9	5-6	108	0.01	0.98	109±2.0	93±1.2	14	107	0.02	0.98	109±0.1	80±1.2	26	Y
10	6-7	102	0.02	0.99	103±1.1	80±1.8	22	105	0.03	0.97	103±1.1	72±2.2	30	Y
11	7-8	104	0.04	0.98	100±1.1	52±1.3	48	114	0.03	0.99	107±2.0	67±2.3	38	Y
12	8-9	108	0.07	0.99	102±1.9	40±2.2	60	112	0.04	0.98	107±2.3	64±1.3	40	Y

DISCUSSION

In all incubations (Table 2) N-removal followed exponential reduction curves. Körner and Vermaat (1998), using lower initial nitrogen concentrations (72-85 mg-N l^{-1}), reported higher nitrogen removal coefficients. This was probably due to the high duckweed biomass per water volume ratio in their 3 to 5 cm deep containers. N-removal in algae systems in our incubations is difficult to compare with values reported in literature for waste stabilisation ponds, which vary from negligible (Silva *et al*., 1987) up to 95% (Middlebrooks *et al*., 1982) depending on system configuration and operational parameters of the ponds.

The major mechanism of N-removal in algae systems was due to biological uptake of nitrogen by dispersed biomass that partly settled or attached to the walls. This is in agreement with Ferrara and Avci (1982) who found that sedimentation was the main nitrogen removal pathway. An increase in attachment and sedimentation was observed at the high pH range of 7-9 probably due to favorable growth conditions for algae biomass. At pH range of 7-9 the non-ionised NH_3 concentration were below the toxicity levels for algae as reported in literature (Konig *et al.*, 1987; Matusiak, 1977). In duckweed systems at pH 5-7, duckweed cover prevented algae development and consequently reduced their attachment to walls and sedimentation. At high pH range (8-9) the duckweed cover was reduced due to inhibition factors allowing for sunlight penetration and algae development. The high pH values also caused sedimentation of decaying duckweed and increased N-accumulation in the sediment.

Ammonia volatilisation increased with increasing pH values. Less duckweed density at pH 8-9 probably resulted in more surface exposed to the atmosphere. This resulted in similar ammonia volatilisation in algae and duckweed systems at higher pH values. The observed ammonia volatilisation rates (Figure 2 and 3) at high pH range are lower than values reported for concentrated slurry and piggery wastes (400-1400 mg-N $m^{-2}d^{-1}$) (Pain *et al*., 1990; Zhang *et al*., 1994; Sommer, 1997). In a study by Shilton (1996), ammonia volatilisation rates were found to proportionally increase with increase in piggery pond nitrogen concentrations. The reported volatilisation at 106 mg-N l^{-1} (average initial concentration in our incubations) compare closely with our experimental results.

Both DO and pH affected the denitrification rates in both algae and duckweed. In anaerobic and aerated incubations, lower denitrification rates were found compared to control incubations. Nitrification-denitrification, which accounted for less than 4% of initial nitrogen content, could occur in the micro-sites of the biofilm attached to the duckweed. Duckweed might transport oxygen to the water through the root zone (Moorhead and Reddy, 1988), and therefore coupled nitrification-denitrification could occur within the biofilm attached to the duckweed. In aerated incubations (Inc. 9-12), the presence of nitrate (6.3-15.5 mg l^{-1}) at the end of the incubation provided evidence for the occurrence of nitrification. The absence of anaerobic conditions made denitrification a limiting step, since its occurrence was limited to anaerobic micro-niches within the containers. In control incubations (Inc. 5-8) denitrification rates were higher at pH values of 8 to 9 compared to rates at pH values of 5 to 8. Since denitrification was high, also nitrification was high. Hunik *et al*., 1992 also found higher nitrification rate at pH of 8-8.5 than rate at pH of 7.5. Azov and Tregubova (1995) reported an even higher optimal pH value (9) for nitrification in stabilisation reservoirs. The denitrification rates measured in control incubations (Inc. 5-9) ranged from 24 to 296 mg-N $m^{-2}d^{-1}$. The rates at pH range of 7 to 9 are comparable to values of 260 mg-N $m^{-2}d^{-1}$ as reported by Vermaat and Hanif (1998). Alaerts *et al*. (1996) and

Körner and Vermaat (1998) reported lower denitrification rates in the range between 10-50 mg-N $m^{-2}d^{-1}$.

The relative growth rate of duckweed was 0.04 d^{-1} and -0.02 d^{-1} at a pH range of 5-8 and 8-9, respectively. At low pH values of 5-8, RGR of duckweed (0.04) was lower than values reported in literature at similar pH values but at lower ammonium concentrations (Alaerts *et al*., 1996; Körner and Vermaat, 1998; Vermaat and Hanif, 1998). The corresponding ammonia concentration at pH range of 5-8 is negligible compared with the toxicity levels reported by Wang (1991) and Clement and Merlin (1995). Therefore, the reduction in RGR could be attributed to ammonium toxicity. This is in agreement with Caicedo *et al*. (2000), who found that both ammonium and ammonia were responsible for reduction in RGR. Al-Nozaily (2001) also found a correlation between lower N-uptake by duckweed and higher ammonium concentration. At higher pH range of 8-9, inhibition of duckweed growth was probably due to the combined effect of ammonium, ammonia and pH toxicity. Despite the low RGR at pH value of 5-8, N-uptake by duckweed was the major N-removal mechanism. At pH range of 8-9, although N-uptake by duckweed was stopped, overall N-removal of the system increased due to increase in sedimentation and attachment.

Zimmo *et al*. (2000) reported lower N-removal by different mechanisms in similar algae and duckweed systems in laboratory incubations at uncontrolled variation of pH and DO, fluorescent light illumination and quiescent conditions. This is not surprising as light intensity and wind effect plays a role in N-removal. For instance, wind agitates the water surface and could enhance ammonia volatilisation. Mixing due to wind can also enhance diffusion of nitrite and nitrate from the aerobic zones in the container to the sediment or anaerobic microsites in the biofilm on the walls. In addition, attached periphyton to the wall of containers and sedimentation were higher in the outdoor incubations compared to laboratory incubations. Higher light intensity in the outdoor incubations could enhance algae growth and oxygen production in algae-based systems and therefore increases N-removal by sedimentation and nitrification-denitrification (Zimmo *et al*., Submitted).

The scale at which the incubations were conducted and the batch operation are likely to affect N-removal and transformations processes. In laboratory batch incubations Al-Nozaily (2001) found that N-removal is a surface related process. Small systems depth of 30 cm used in our incubations resulted in high surface area per volume ratio. This could enhance the surface related processes such as ammonia volatilisation denitrification and sedimentation. Therefore extrapolating values from batch incubations to real scale or even pilot-scale steady state treatment systems should be carefully interpreted.

CONCLUSIONS

This study was conducted to investigate the effect of dissolved oxygen and pH on both overall nitrogen removal and on N-removal by different mechanisms in algae-based and duckweed-based wastewater systems. Results showed that N-removal by different mechanisms in both systems are more dependent on the pH variations than on the oxygen variations. At high pH ranges (7-9) at both anaerobic and aerobic conditions, N-removal was significantly higher in comparison with low pH ranges (5-7) in both systems due to increase in sedimentation and ammonia volatilisation. Significantly higher N-removal in duckweed system (26-33%) than algae system (14-24%) was found at lower pH range of 5-7. The higher removal in duckweed system was mainly due to duckweed uptake. At high pH values (7-9), the increase in N-removal by

sedimentation in algae system and the decrease in N-uptake by duckweed in duckweed system resulted in significantly higher N-removal in algae system (45-60%) than duckweed system (38-41%).
An increase in N-removal by 17-20% in the control incubations of algae systems occurred at high pH range (7-9) due to increase in denitrification and sedimentation compared with removal at anaerobic and aerobic incubations. In duckweed systems the increase was 22-25%.

ACKNOWLEDGEMENT

The authors are grateful to the Dutch government for financially supporting this research within the collaboration project (WASCAPAL) between Birzeit University, West Bank and the UNESCO-IHE Institute for Waster Education, The Netherlands. The first author wishes to thank Saleem Al-Yahya (MSc. Student) and the laboratory technicians Abeer Al-Sous, Abdelhaleem Al-Fouqaha and Marwan Ayad for their contribution in conducting this work.

REFERENCES

Alaerts G.J., Mahbubar M.R., Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res.* **30**: 843-852.

Al-Nozaily F. (2001). *Performance and process analysis of duckweed-covered sewage lagoons for high strength sewage*. The case of Sana'a Yemen. Ph.D. Thesis, IHE, The Netherlands.

APHA (1992). Standard methods for examination of water and wastewater. 18th edition, *Am. Publ. Assoc*., Washington D.C., USA.

Azov Y. and Tregubova T. (1995). Nitrification processes in stabilisation reservoirs. *Wat. Sci. Tech.* **31**: 313-319.

Caicedo J. R., van der Steen N.P. Arce O. and Gijzen H.J. (2000). Effect of total nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Wat. Res.* **15**: 3829-3835.

Clement B. and Merlin G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ*. **170**: 71-79.

Ferrara R.A. and Avci C.B. (1982). Nitrogen dynamics in waste stabilisation ponds. J. *WPCF*. **54**: 361-369.

Gijzen H.J. (2001). Anaerobes, aerobes and phototrophs: A winning team for wastewater management. *Wat. Sci. Tech.* **44**: 123-132

Hunik H., Tramper J. and Meijer H.J.G. (1992). Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentrations. *Appl. Microbiol. Biotechnol*. **37**: 802-807.

Hunt R. (1978). Plant growth analysis. Edward Arnold, London. 67 pp.

Konig A., Pearson H. P., Silva S. A. (1987). Ammonia toxicity to algal growth in waste stabilisation ponds. *Wat. Sci. Tech.* **19**: 115-122.

Körner S. and Vermaat J. E. (1998). The relative importance of *Lemna gibba* L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Wat. Res.* **32**: 3651-3661

Matusiak K. (1977). Studies on purification of wastewater from the nitrogen fertiliser industry by intensive algal cultures. *Acta Microbiologica Polonica*. **25**: 233-242.

McLay C.L. (1976). The effect of pH on the population growth of three species of duckweed: *Spirodela ologorhiza, Lemna minor and Wolffia arrhiza*. *J. Freshwater Biol*. **6:** 125-136.

Middlebrooks E.J., Middlebrooks C.H., Reynolds J.H., Watters G.Z., Reed S.C. and George D.B. (1982). *Wastewater stabilisation lagoon design, performance and upgrading*. Macmillan, NY, USA.

Moorhead K.K. and Reddy K.R. (1988). Oxygen transport through selected aquatic macrophytes. *J. Environ. Qual.* **17:** 138-142.

Novozamsky I., Houba V.J.G., Van Eck R. and Van Vark W. (1983). A novel digestion technique for multi-element plant analysis. *Comm. Soil. Sci.Plant. Anal.* **14:** 239-248.

O'Brien J.W. (1981). Use of aquatic macrophytes for wastewater treatment. *J.Env. Eng. Div.*, ASCE. **107:** 681-698.

Pain B.F., Misselbrook T.H., Clarkson C.R. and Rees Y.J. (1990). Odour and ammonia emissions following the spreading of anaerobically-digested pig slurry on grassland. *Biological Wastes*. **34**: 259-267.

Porath and Pollock (1982). Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquat. Bot*. **13:** 125-131.

Reed S.C. (1985). Nitrogen removal in stabilisation ponds. *J.WPCF*. **57:** 39-45.

Shilton A. (1996). Ammonia volatilisation from a piggery pond. *Wat. Sci. Tech*. **33:** 183-189.

Silva S.A., de Pliveira R., Soares J., Mara D.D. and Pearson H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Wat. Sci. Tech*. **31:** 321-330.

Silva S.A., Mara D.D. and de Oliveira R. (1987). The performance of five deep waste stabilisation ponds in northeast Brazil. *Wat. Sci. Tech.* **19:** 61-64.

Skillicorn P., Spira W., Jouney W. (1993). Duckweed Aquaculture, a new aquatic farming system for developing countries. The World Bank, Washington, USA.

Sommer S.G. (1997). Ammonia volatilisation from farm tanks containing anaerobically digested animal slurry. *Atmospheric Environment*. **31:** 863-868.

Vermaat J. E. and Hanif K.M. (1998). Performance of common duckweed species (*Lemnaceae*) and waterfern (*Azolla filiculoides*) on different types of wastewater. *Wat. Res.* **32:** 2569-2576.

Wang W. (1991). Ammonia toxicity to macrophytes (Common duckweed and rice) using static and renewal methods. *Environm. Toxic. Chem*. **10:** 1173-1178.

Zhang R.H., Day D.L., Christianson L.L. and Jepson W.P. (1994). A computer model for predicting ammonia release rates from swine manure pits. *J. Agric. Engng Res*. **58:** 223-229.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2000). Comparison between algae-based and duckweed-based wastewater treatment: differences in environmental conditions and nitrogen transformations. *Wat. Sci. Tech*. **42:** 215-222.

Zimmo O. R., van der Steen N. P. and Gijzen H. J. (Submitted). Quantification of nitrification and denitrification rates in algal and duckweed based wastewater treatment systems.

Zirschky J. and Reed S.C. (1988). The use of duckweed for wastewater treatment. *J.WPCF*. 60: 1253-1258.

Chapter 3

COMPARISON OF HYDRAULIC FLOW PATTERNS IN ALGAE AND DUCKWEED-BASED CONTINUOUS FLOW PILOT-SCALE WASTE STABILISATION PONDS

Submitted as:

Zimmo O. R., Al-Ahmed M., van der Steen N. P., Awuah E., Caicedo J. and Gijzen H. J. Comparison of hydraulic flow patterns in algae and duckweed- based continuous flow pilot-scale waste stabilisation ponds. *Wat. Res*.

Chapter 3

COMPARISON OF HYDRAULIC FLOW PATTERNS IN ALGAE AND DUCKWEED- BASED CONTINUOUS FLOW PILOT-SCALE WASTE STABILISATION PONDS

ABSTRACT

The hydraulic characteristics of two sets of pilot-scale sewage stabilisation ponds, one located in Palestine and another in Colombia were studied. The pond system in Palestine consisted of two treatment lines of algae and duckweed-based ponds each with four ponds in series and a retention time of 7 days per pond. In Colombia an algae and a duckweed-based pond system were operated in parallel, but now each system consisted of just one pond. Actual retention time in the algae and duckweed pond was 21 and 22 days, respectively. Hydraulic behaviours of stabilisation ponds were studied by dosing lithium chloride (LiCl) tracer at the inlets of treatment lines. LiCl concentrations at the outlet were measured for 58 and 39 days for the treatment plants in Palestine and Colombia, respectively. The hydraulic characteristics calculated from the tracer response curve at the effluents of each pond were evaluated. The tracer study of the Palestine system demonstrated that hydraulic characteristics were similar in both ABPs and DBPs. Actual retention times were observed to be higher than the theoretical retention time resulting in negative dead space values. The tracer peak was detected at 74-96% of the theoretical retention times indicating limited short-circuiting. The dispersion number and time-concentration curves of Li indicate that the hydraulic behaviour of the ponds was neither a completely mixed nor a plug flow but dispersed flow. Using available equations in literature gave negative dead space values. The tracer experiment on the larger single pond system in Colombia showed similar actual retention times, which was less than the theoretical retention times but less short-circuiting and more plug flow-like conditions in the duckweed pond compared to the algae pond. Dead spaces of 30% were observed for the algae and duckweed-based ponds. The better hydraulic behaviour of the pond with duckweed cover may be explained by reduced wind-induced short circuiting and reduced mixing caused by the absence of gas bubbling associated with photosynthetic activities in algae ponds. This may lead to a better treatment performance in DBPs than ABPs.

Keywords: Algae, duckweed, hydraulic characteristics, Lithium chloride, stabilisation ponds, tracer

INTRODUCTION

Waste stabilisation ponds (WSPs) are appropriate for wastewater treatment in the tropics. WSPs represent a feasible wastewater treatment option for areas where the climate is favourable and land is available (Shelef and Kanarek, 1995). An advantage of WSPs compared to other technologies such as activated sludge is that besides suspended solids and COD, also pathogens are removed.

The design of WSPs is based on two concepts. First: assuming first order kinetics for the removal of both coliform bacteria and organic matter (i.e. BOD and COD). Second: the flow pattern in the ponds, which depends on various factors. Some researchers assumed completely mixed flow conditions (Mara and Silva, 1979; Ferrara and Harleman, 1980) while others considered plug flow conditions (Polprasert *et al*., 1983). In reality, neither a completely mixed nor a plug flow conditions is encountered but

usually dispersed flow condition has been found (Wehner and Wilhelm, 1956). The completely mixed and plug flow conditions describe ideal flow conditions and the dispersed flow is non-ideal flow (Agunwamba *et al*., 1992; Levenspiel, 1983).
Duckweed-based ponds (DBPs) are WSPs with a cover of floating duckweed plants (*Lemnaceae*). The presence of a duckweed cover in DBPs may influence the hydraulic characteristics in this system compared with conventional algae WSP with uncovered surface providing conditions for algae colonisation (ABP). The presence of a duckweed cover may affect hydraulic characteristics by reducing the wind effect. This may reduce short-circuiting resulting in better plug flow conditions and therefore, wastewater retention in the pond may approach theoretical retention time (t = total pond volume/inflow). Hydraulic behaviour in WSPs will have a great influence on pond performance. Despite the importance of this parameter, no studies have been dedicated to comparison of hydraulic characteristics of algae versus macrophytes covered WSPs. Therefore, the present study aims to assess possible differences in hydraulic characteristics in both systems in terms of the actual retention time and short-circuiting behaviour in addition to the type of flow as indicated by measurement of the dispersion number. The hydraulic characteristics in each stage of the duckweed-based and algae-based stabilisation pond treatment systems were evaluated via tracer (lithium chloride) pulse experiments.

Theoretical Background

Flow in ponds could not be described as ideal plug flow or complete mixed flow but usually as a non-ideal flow pattern. Levenspiel (1972) described two methods for the characterisation of non-ideal flow pattern. The tanks-in-series model, which describes the flow pattern by indicating the number of CSTR reactors in series (N) that would result in the same retention time distribution. The dispersion model quite satisfactorily represents a flow that deviates in some extent from plug flow. Each model is a one-parameter model. The parameters of both models are calculated from the measured actual retention time ($\bar{t}$) (the centroid) and the variance (σ^2) (the spread). The variance of the time-concentration curve describes the distribution of effluent leaving the pond.

$$\bar{t} = \frac{\int_0^\infty tC\,.dt}{\int_0^\infty C\,.dt} \tag{1}$$

$$\sigma^2 = \frac{\int_0^\infty t^2 C dt}{\int_0^\infty C dt} - \overline{t^2} \tag{2}$$

where, C = tracer concentration (mg l^{-1})
t = time (hours)

The retention time distribution curve is often shown as the normalised effluent tracer concentration and normalised time.

$$\text{Normalised concentration} = \frac{C}{Co} \tag{3}$$

where, C_o = amount of tracer per reactor volume.

$$\text{Normalised time} = \frac{t}{HRT_{th}} \qquad (4)$$

where, HRT_{th} = Theoretical retention time (Volume/flow rate) (hours).

For the tanks in series model:

$$N = \frac{\overline{t^2}}{\sigma^2} \qquad (5)$$

If $N = 1$, the flow pattern is complete mixing and if $N = \infty$, the flow pattern is plug flow.

For the dispersion model:
The dispersion number is a measure of the amount of mixing.

$$d = (\frac{D}{uL}) \qquad (6)$$

where, D = dispersion coefficient in ($m^2\ s^{-1}$)
u = average velocity in m s^{-1}
L= length in m from inlet to outlet
If d = zero, flow pattern is plug flow and if d = 1, the flow pattern is complete mixing.
The dispersion number is calculated as follows:

$$d = 0.5\ \frac{\sigma^2}{\overline{t^2}} \qquad (7)$$

The fraction of dead-space (stagnant pockets) is usually calculated as follows:

$$\text{Dead-space} = (1 - \frac{\bar{t}}{HRT_{th}}) \times 100\% \qquad (8)$$

Another important index that describes the mixing behaviour in the non-ideal flow pattern is the index of short-circuiting (α_s).

$$\alpha_s = \frac{\bar{t} - t_p}{\bar{t}} \qquad (9)$$

where, t_p = time to reach peak or maximum concentration.

In case α is zero there is no short-circuiting. When there is a large extent of short-circuiting the index will approach the extreme value of 1 (Kilani and Ogunrombi, 1984).

MATERIAL AND METHODS

Pilot Plant Layout and operation

Palestine:
The tracer experiment was carried out in Palestine in a pilot-scale pond system (Figure 1) at the new campus of Birzeit University (BZU), which is 26 km to the north of Jerusalem.

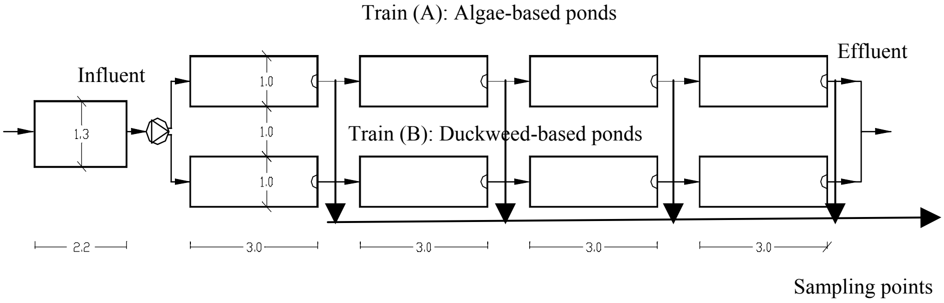


Figure 1. Schematic diagram of the pilot plant in Palestine showing the sampling points used in determining time-concentration plots across both algae and duckweed-based pond systems

The pilot plant consisted of a holding tank with the dimensions: 2.2 m length, 1.3 m width and 1.9 m depth. The holding tank is followed by two parallel systems: duckweed-based ponds (DBPs) and algae-based ponds (ABPs). Each system consisted of four equal ponds connected in series. The cross-sectional dimensions of each pond are: 1m width, 3m length and 0.9 m depth. The inlet pipes in the first pond of the DBPs and ABPs systems are placed 20 cm below the water surface. Baffles of 10 cm below the water surface are constructed at the outlet of each pond to avoid short-circuiting of influent to effluent and to avoid transfer of floatable materials to the consecutive ponds. The pilot plant was operated as a continuous flow system. The source of wastewater was raw sewage from Birzeit University treatment plant, which receives its sewage from the University new campus. The wastewater from the holding tank of 2 day retention time was pumped equally through peristaltic pump to the first algae and first duckweed pond. The flow rate (Q) of the pump was 0.38 cubic meters per day for every pond system. Further water transport to the subsequent ponds in each system was occurring via gravity. A hydraulic retention time (HRT) of seven days and water depth of about (0.9) meter was maintained in each pond. The volume of each pond was 2.7 cubic meters (3 m length, 1 m width and 0.9 m depth).

Colombia

The tracer experiment was conducted in a continuous flow pilot plant that consisted of an algae and duckweed-based pond (*Spirodela polyrrhiza*) operated in parallel and receiving effluent from a UASB reactor in Ginebra, Colombia (Figure 2). The dimensions of each pond were 64 m length, 4.95 m (algae pond) and 5.2 (duckweed pond) width, and 0.7 m depth. The L/W ratio was 12.8. Flow rate of 16.6 m^3 d^{-1} was maintained in each pond. The theoretical retention time was 13.5 days.

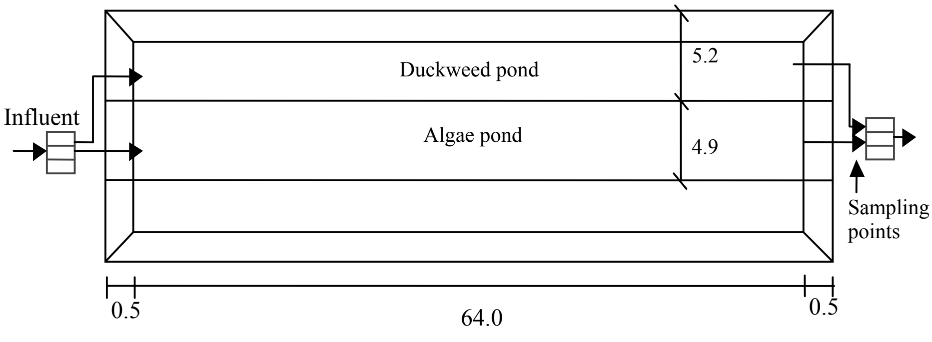


Figure 2. Schematic diagram of the treatment plant at Ginebra, Colombia. All dimensions are in meter.

Tracer experiment procedures

Palestine
The tracer experiment was conducted during the winter season (from February to April 2000) in Palestine. Output samples were taken for a period of 56 days (twice the theoretical residence time). Ponds hydraulic behaviour was studied according to a tracer pulse-injection technique using Lithium chloride (LiCl). Lithium was chosen because it has low toxicity, low detection level and minimum adsorption properties, in addition to the lithium chloride (LiCl) density, which may be the same as the wastewater density. An amount of (120 g) of lithium chloride (LiCl) was dissolved in one litre of distilled water and added for each pond system to achieve a concentration of 11.1 mg l^{-1}. This concentration corresponds to a concentration of 7.28 mg Li l^{-1} (44.4 mg LiCl l^{-1}) in the first pond. During the experimental periods, the water balance was determined. Flow rate was kept at 0.38 m^3 d^{-1} in both ABPs and DBPs. Inflow was monitored by recording daily the water level in the holding tank. Clogging that might have occurred during one day was compensated by pumping extra the next day. Grab samples of about 100 ml were collected in a clear plastic container for 56 consecutive days (two times the theoretical retention time) from the effluent of each pond of the ABPs and DBPs systems. Higher sampling frequency (5 times per day) was used during the period when rapid changes in concentration were detected and reduced to twice per day afterwards. Collected samples were paper filtered and stored in the refrigerator prior to analysis. The maximum storage time for the samples was two days. Lithium was analysed on Perkin-Elmer Model XL100 Atomic Absorption Spectrometer (APHA, 1995). Three lithium chloride samples of 3, 9 and 18 mg l^{-1} were prepared as standards for calibration of the device. Initial background of tracer concentration in raw sewage and pond water was measured in all sampling points immediately before the addition of the tracer pulse.

Colombia
The tracer experiment in Colombia was conducted from 30 October to 15 December 2000. Regulation of flow rate was checked periodically by measuring flows at the inlets five times a day. Average flow rate was calculated accordingly. Sampling of the tracer from the outlet was done four times a day for 47 days 3.5 times the retention period of 13.5 days. Tracer solution was prepared by adding 2.5 kg into 5 l of water and completely dissolving it. The solution was equally divided for the two ponds and pulse-injected at the inlets simultaneously. This gave an average concentration of 8.9 mg LiCl l^{-1} (1.5 mg Li l^{-1}) in the total pond volume. Lithium was analysed on Perkin-Elmer Model 5000 Atomic Absorption Spectrometer (APHA, 1995).

RESULTS

Palestine
The background lithium concentration in the influent and effluents of the ABPs and DBPs was found to be zero. The time-concentration curves at the effluents of the first, second, third and fourth algae ponds (a) and the corresponding duckweed ponds (b) are shown in Figure 3. Similar single-peak time-concentration curves expected of dispersed flow model are exhibited at the effluents of each pond of the algae and duckweed-based systems. In all cases the time-concentration curves were characterised by an unsymmetrical curve with extended tail. Measurable retention of the tracer was more than 4 times the hydraulic retention time.

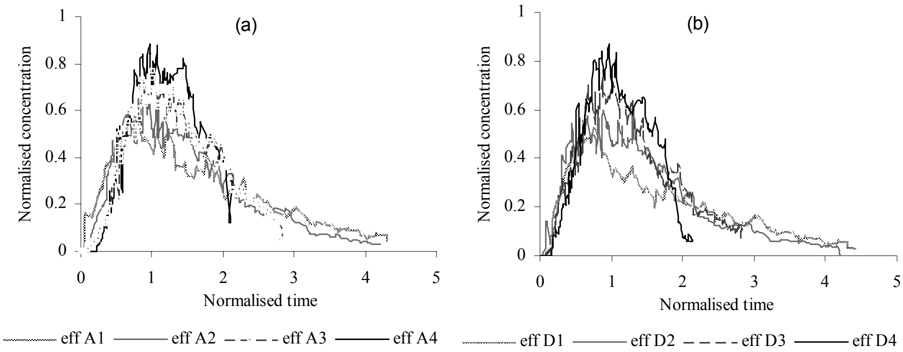


Figure 3. Time-concentration curves at the four effluent points across the algae (a) and duckweed-based systems (b) in Palestine.

The hydraulic characteristics of the flow pattern at the effluents of each pond in the ABPs and DBPs systems are summarised in Table 1. The actual retention times obtained using the residence time distribution technique exceeded the computed theoretical values, which resulted in negative values for dead spaces. In the four effluent points across the line of the ABPs and DBPs systems, the peak tracer concentration was observed at 0.74, 0.86, 0.88 and 0.96 of the theoretical detention time, respectively suggesting the occurrence of short-circuiting which decreased as the number of ponds in series increased. This is also indicated by the index of short-circuiting. It is not surprising for more short-circuiting to occur in a single pond system. The ponds dispersion numbers (Table 2) fall between an intermediate and large amount of dispersion (Levenspiel, 1972). A dispersion number of 0.20 nd 0.22 n the first algae and duckweed-based ponds respectively implies a large amount of dispersion approaching a well-mixed system. Decrease of dispersion occurred as number of ponds increased. A good degree of tracer recovery at every stage in both algae and duckweed system (84-99%) was achieved.

Table 1. Hydraulic characteristic in algae and duckweed-based pond series.

Parameter	Algae-based ponds system				Duckweed-based ponds system			
	1 pond	2 ponds	3 ponds	4 ponds	1 pond	2 ponds	3 ponds	4 ponds
HRT_{th} (days)	7.0	14.0	21.0	28.0	7.0	14.0	21.0	28.0
t (days)	11.4	19.3	29.1	34.1	11.1	18.1	26.5	31.5
σ^2	0.40	0.25	0.19	0.13	0.44	0.28	0.24	0.16
N	2.52	4.02	5.21	7.87	2.26	3.59	4.19	6.36
d	0.20	0.12	0.10	0.06	0.22	0.14	0.12	0.08
dead space (%)	-63	-38	-38	-22	-59	-29	-26	-12
α_s (-)	0.54	0.36	0.27	0.21	0.52	0	0.32	0.14
Li-recovery (%)	98	94	99	96	85	84	91	88

Colombia

The hydraulic characteristic of the algae and duckweed-based ponds in Colombia are summarised in Table 2. Significant differences in terms of percentages of the hydraulic characteristics in the algae and duckweed-based pond were observed. (Table 2). Approximately, 60% of the tracer was observed to pass through the outlet of each pond. The loss in tracer could be only explained by the seepage through the embankment (where applicable) and percolation through the bottom. Assuming 100% tracer recovery, theoretical hydraulic retention times for water that stays in the ponds were 23 and 22 days in the algae and duckweed-based pond, respectively. The actual retention times (16.6 days in the algae pond and 15.3 days in the duckweed pond) were therefore less than the theoretical retention times. Dead spaces of 28 and 30% were obtained with other hydraulic parameters remaining the same as in Table 2. Hydraulic behaviour in terms of retention time in both ABPs and DBPs was similar. However, higher number of stirred tanks in series and lower dispersion number was found in the duckweed-based pond suggesting better plug flow behaviour in comparison with algae-based pond. Index of short-circuiting was also smaller in duckweed pond compared with algae pond.

Table 2. Hydraulic characteristics of the algae and duckweed-based ponds in Colombia.

Parameter	HRT (hrs)	t (hrs)	σ^2 (-)	N (-)	D (-)	Dead space (%)	α_s (-)	Li recovery (%)
Algae-based pond	13.5	16.6	0.41	2.4	0.21	-22	0.57	63
Duckweed-based pond	13.5	15.3	0.31	3.4	0.16	-13	0.38	60

The time-concentration curves for the algae and duckweed-pond after correction for seepage loss are shown in Figure 4. A smooth curve was observed for the duckweed-based pond and the maximum concentration was observed at 32% of the theoretical retention time. While for algae-based pond irregular curve and three peaks at 6, 13 and 28% of the theoretical retention time were observed (Figure 4) suggesting the occurrence of severe short-circuiting.

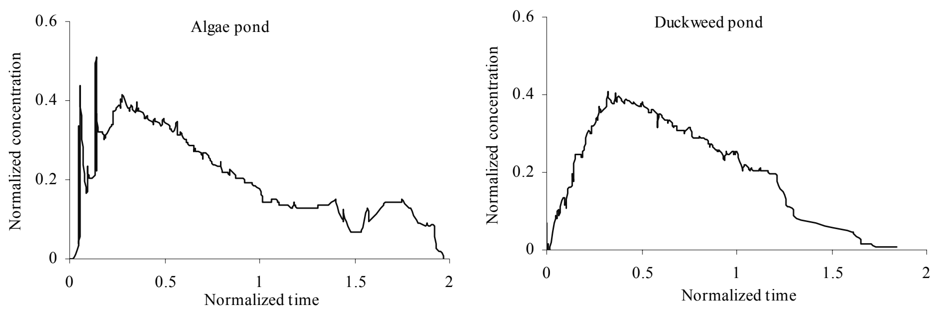


Figure 4. Time-concentration curve of the algae and duckweed-based ponds in Colombia.

DISCUSSION

The time-concentration curves of the tracer study at the pilot-scale system in Palestine (Figure 3) showed a single peak as expected of a dispersed flow. Despite the fact that the tracer experiment was conducted during the winter period no multiple peaks were observed. This is in contrast with other authors (Mangelson and Watters, 1972; Marecos do Monte and Mara, 1987; Moreno, 1990), who found multiple peak curves. They concluded that the multiple peaks found in winter could be due to the superposition of circulatory flow patterns due to wind effects.

The time-concentration curve of the tracer experiment for the series of ponds culminated at the first, second, third and fourth ponds shows an extended tail which indicates a shift in the actual retention time. Therefore, the actual retention time became bigger than the theoretical retention time resulting in surprisingly negative values for the dead space. Rationally, the actual retention time should be smaller than the theoretical retention time due to inactive pond volume comprised of sludge at the bottom of the pond and stagnant regions within the ponds formed as a result of pond geometry. Similar contradictory results of tracer study have been found (Kilani and Ogunrombi, 1984; Thackston *et al*., 1987). They explained this phenomenon by the diffusion of the tracer during the early stages of the flow in the dead spaces, which diffuses out in the latter stages. This will cause the time-concentration curve to be excessively elongated and leading to a shift in the centroid resulting in a higher value for the actual retention time. Also, because of the higher density of LiCl solution compared to pond wastewater density, LiCl probably passed onto the bottom of the pond and slowly dissolved into pond water where it leached out at a much longer period and thus giving spurious tracer curves. It can be concluded that the formula used for the calculation of the degree of dead space is not applicable in our pond systems. The thinner sediment layer in duckweed ponds compared to algae ponds (Zimmo *et al*., 2000) could explain the less negative dead spaces in comparison with algae ponds. Sampling for Li determination from the effluents of ponds 1, 2, 3 and 4 in both systems was terminated after 4.3, 4.1, 2.8 and 2 theoretical retention times of their series, respectively. The higher sampling time at effluents of pond 1 and 2 resulted in elongated tails that shifted the actual retention time to the right in the time-concentration curves. This led to higher negative dead spaces observed in these two ponds in comparison with the rest of ponds where sampling lasted for only 2 to 3 times the theoretical retention time.

Net inflow (actual inflow + rainfall - evaporation) into the pond systems was calculated elsewhere (Zimmo *et al*., Submitted) and found to be slightly higher than the actual flow rate due to rainfall. However this small increase would result only in negligible effects on the hydraulic parameters calculated for both systems. Hydraulic parameters could be affected in systems where inflow reduces along the system due to evaporation.

It was hypothesised that the duckweed cover would mitigate the wind effect and therefore reduce mixing. This hypothesis could be true if a dense duckweed cover is maintained. However, comparable dispersion numbers were found in both ABPs and DBPs in Palestine. The mixing and molecular diffusion occurred in ponds due to duckweed harvesting and the reduced growth of duckweed during the winter season when the tracer experiment was conducted, resulting in similar conditions as if the duckweed cover was not present. The wind effect and rainfall also disturbed the duckweed cover and caused mixing to occur in both algae and duckweed-based ponds.

A higher number of stirred tanks in series (N) when the number of ponds increases across the line of treatment is expected due to the increase in L/W ratio, which led to a mixing behaviour with better plug flow behaviour (Kilani and Ogunrombi, 1984). The lower L/W ratio in the first algae and duckweed ponds resulted in the increase of the extent of short-circuiting and lower N-value. The N-values increased significantly where the L/W ration increased across the line of treatment. This is in agreement to some results found in literature (Kilani and Ogunrombi, 1984).

Short-circuiting in our systems was limited. Pedahzar *et al*., (1993) observed sever short-circuiting that was caused by thermal stratification, which was not occurring during winter season in our system. Other factors such as wind effect and inlet-outlet arrangements could be responsible for the observed short-circuiting. The tracer result during the summer period is expected not to vary much from that of the winter period for the ABPs and DBPs systems in Palestine, since also during summer the stratification effect is not likely to occur in such small ponds. The water temperature in raw sewage compared with the temperature profile through the ponds showed a difference of a maximum of 1 ^{o}C (Zimmo *et al*., 2000). Temperature can influence the hydraulic performance of ponds of deeper depth of 2 to 3 m, which often thermally stratify, which significantly increases their percentage of dead space (Drakides, 1987; Uhlmann, 1979). It is expected that the well-established duckweed cover during the summer period would reduce the disturbance in water column due to wind effect. However, the long hydraulic retention time would promote axial dispersion and back and forth mixing which will neutralise the influence of duckweed cover. Also, the harvesting practice in the ponds of such a small area would result in a disturbance in the water column exhibiting differences in hydraulic behaviour between DBPs and ABPs of minor importance.

As the hydraulic characteristics of the ABPs and the DBPs do not change significantly, the differences in the treatment performance of the two systems (ABPs and DBPs) observed (Zimmo *et al*., 2002) therefore would be due to other factors rather than the differences in the hydraulic behaviour.

In full-scale ponds in Colombia, the tracer experiment revealed clear differences in hydraulic behaviour in algae and duckweed-based ponds in terms of mixing behaviour and short-circuiting. But, similar actual retention times in both ponds, which was less than the theoretical retention times were found. The duckweed cover maintained quiescent conditions and reduced wind-induced short-circuiting. This in its own right results in a hydraulic behaviour with better plug flow behaviour. Also, It was visualised that the well-established duckweed cover formed a physical barrier where surface movement and short-circuiting caused by wind was inhibited. Contrary, in algae-based pond, produced oxygen due to photosynthesis of algae biomass during daytime caused mixing in the water column to occur. Besides, the movement of alga as moving microorganisms might exert a good degree of mixing in the water column. The water surface of ABP was observed visually to move fast from the inlet towards the outlet causing clear short-circuiting. The time concentration curve in the duckweed pond is an indicator of little disturbance due to the duckweed cover (Figure 3). While multiple peaks was observed within less than 10% and 25% of the theoretical retention time in the algae pond, suggesting significant short-circuiting. Better plug flow behaviour and less short-circuiting was found in the duckweed ponds when comparing the number of stirred tanks in series (model) and the short-circuiting index of the two ponds (Table 2). Dispersion number was also lower in the duckweed pond than in the algae pond. This shows that in spite of the harvesting practice in duckweed pond, duckweed cover was able to maintain better plug flow behaviour and less turbulence in the water column. The presence of duckweed cover in the duckweed pond resulted in higher number of stirred tanks in series.

Less percentage of Li recovery was observed in the experiment in Colombia (Table 2) in comparison with the Palestine experiment (Table 1). This was probably due to seepage, which was not prevented in the earthen ponds in Colombia.

Despite the four fold higher value of L/W in the algae pond in Colombia in comparison with the first algae pond in Palestine, a similar *N* value was obtained. A small increase of *N* for L/W ratio > 10 is considered high enough to create better plug flow behaviour

(Thackston *et al*., 1987). However, significantly higher *N* value was obtained for equivalent L/W ratio ponds in Palestine than in Colombia probably because the length for the Palestine system is divided into four ponds in series.
The differences in hydraulic characteristics of the full-scale algae and duckweed pond in Colombia demonstrated that duckweed cover significantly improves the hydraulic behaviour in pond systems. Short-circuiting is less and better plug flow behaviour prevailed in the pond with duckweed cover. Effect of the difference in hydraulic behaviour of the two ponds on its performance is currently investigated. However, similar hydraulic behaviour was observed in the ABPs and DBPs in Palestine during winter season and this condition is not expected to change during the summer period. It may be concluded that the hydraulic behaviour of the pond system could be site specific due to differences in system configuration, arrangement of inlets and outlets and wastewater characteristics. Preferably, hydraulic characteristic should be determined for modelling and predicting pond performance.

CONCLUSIONS

Based on the results obtained from the tracer experiment to characterise the hydraulic behaviour in the ABPs and DBPs, the following may be concluded:
Palestine: The hydraulic characteristics for each pond's retention time with respect to previous ponds in series were evaluated. The tracer study demonstrated that hydraulic characteristics were similar in both ABPs and DBPs. It was expected that the duckweed cover would reduce wind mixing. However, harvesting practice and long hydraulic retention times promoted axial dispersion and back and forth mixing which neutralised the influence of the duckweed cover. Actual retention times were observed to be higher than the theoretical retention times in both systems. This suggests that the higher density of LiCl solution compared to pond wastewater density, probably caused LiCl to pass onto the bottom of the pond and slowly dissolved into pond water where it leached out at a much longer period and thus giving spurious tracer curves. The tracer peak was detected at the outlets of various ponds at 74-96% of the theoretical retention times with lesser value at initial ponds indicating limited short-circuiting decreases as the number of ponds increases in series. The dispersion number and the time-concentration curves indicate that the hydraulic nature of the ponds is dispersed-plug flow. Using available equations in literature for calculating dead space yielded negative values suggesting that the available equation in literature is not suitable and need further investigation. The observed differences in treatment performance of the two systems (Zimmo *et al*., 2002) would be due to other factors rather than differences in the hydraulic behaviour.
Colombia: The tracer experiment on larger single pond systems in Colombia showed less short-circuiting and better plug flow behaviour in the duckweed pond than in the algae pond. In the duckweed pond, the well-established duckweed cover and the short retention time may reduce wind-induced short-circuiting and produce nearly a plug flow. The effect of differences in hydraulic performance in the two ponds system on efficiencies is currently subject to investigation.

ACKNOWLEDGEMENTS

This work was financed by the Netherlands Government within the scope of the WASCAPAL project in Palestine and ESEE project in Valle University Cali-Colombia.

These projects are in cooperation with UNESCO-IHE Institute for Waster Education, The Netherlands. The authors are grateful to Barry Lloyd and Andy Shilton for helpful discussions.

REFERENCES

Agunwamba J.C., Egbuniwe N., Ademiluyi J.O. (1992). Prediction of dispersion number in waste stabilisation ponds. *Was. Res*. **26**(1):85-89.

Drakides C. (1987). Le lagunage naturel, mecanismes et performances. In *Inter-University Seminar on Wastewater treatment in Waste Stabilisation Ponds and Aerated Lagoons*, ed P. Vander Borght and J.-L. Vasel, pp. 1-24. F.U.L., Arlon (Belgium).

Ferrara R.A. and Harleman D.R.F. (1980). Dynamic nutrient cycle model for waste stabilisation ponds. *J.Env. Engrg. Div., ASCE*, 106(1), 37-53.

Kilani, J.S. and Ogunrombi J.A. (1984). Effects of baffles on the performance of model waste stabilization ponds. *Wat. Res*. 18(8): 941-944

Levenspiel, O. (1972). Chemical Reaction Engineering, Second Edition. John Wiley & Sons, New York, NY.

Levenspiel, O. (1983). Chemical Engineering. Department of Chemical Engineering, Oregon State University. 587 p.

Mangelson K.A. and Watters G.Z. (1972). Treatment efficiency of waste stabilisation ponds. *J. San. Engerg. Div., ASCE*. 98:407-425.

Mara D.D. and Silva S.A. (1979). Sewage treatment in waste stabilisation ponds: recent research in northeast Brazil. *Prog. Wat. Techn. IAWPR*, 11(1/2), 341-344.

Marecos do Monte M.H.F. and Mara D.D. (1987). The hydraulic performance of waste stabilisation ponds in Portugal. *Wat. Sci. Tech*. 19: 219-227.

Moreno D. (1990). A tracer study of the hydraulics of facultative stabilisation ponds. *Wat. Res*. 24: 1025-1030.

Pedahzar R., Nasser A.M., Dor I., Fattal B. and Shuval H.I. (1993). The effect of baffle installation on the performance of a single-cell stabilisation pond. *Wat. Sci. and Tech*. 27:45-52.

Polprasert C., Dissanayake M.G. and Tanh N.C. (1983). Bacterial die-off kinetics in waste stabilisation ponds. *J. WPCF*. 55(3), 285-296.

Shelef G. and Kanarek A. (1995). Stabilisation ponds with recirculation. *Wat. Sci. Tech*. **31**: 389-397.

Thackston E.L., Shields F.D. and Schroeder P.R. (1987). Residence time distributions of shallow basins. *J. Env. Engrg. Div. ASCE*, 113: 1319-1332.

Uhlmann D. (1979). BOD removal rates of waste stabilisation ponds and hydraulic flow pattern. *Wat. Res*. 13, 193-200.

Wehner J.F. and Wilhelm R.H. (1956). Boundary conditions of flow reactor. *Chem. Eng. Sci*., 6, 89-93.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2000). Nitrogen transformation and wastewater treatment efficiency in algae-based and duckweed-based stabilisation ponds. *Conference proceeding "Managing Water and Waste in the New Millennium"*.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2002). Process performance assessment of algae-based and duckweed-based wastewater treatment systems. *Wat. Sci. Tech*. **45,** 91-101.

Zimmo O.R., van der Steen N. P. and Gijzen H. J. (Submitted). Nitrogen Mass balance for pilot scale algae and duckweed-based waste stabilisation ponds.

Chapter 4

GENERAL PROCESS PERFORMANCE ASSESSMENT OF PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

Adapted from:

Zimmo O. R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. Process performance Assessment of algae-based and duckweed-based wastewater treatment systems. *Wat. Sci Tech*. 45(1): 91-101, 2002.

Chapter 4

GENERAL PROCESS PERFORMANCE ASSESSMENT OF PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

ABSTRACT

A pilot plant experiment was carried out to assess differences in environmental conditions and treatment performance in two systems for wastewater treatment: algae-based ponds (ABPs) and duckweed-based (*Lemna gibba*) ponds (DBPs). Each system consisted of a sequence of 4 equal ponds in series and was fed with a constant flow rate of partially treated wastewater from Birzeit University. Physico-chemical parameters and the removal of organic matter, nutrients and faecal coliforms (FC) were monitored within each treatment system over a period of 12 months. The results show clear differences in the environmental conditions. In ABPs significantly ($P<0.05$) higher pH and DO values were observed than in DBPs. DBPs were more efficient in removal of organic matter (BOD and TSS) than ABPs. The FC reduction was higher in ABPs. However, the quality of the effluent from the third and fourth duckweed pond (total retention time of 21 and 28 days) did not exceed the WHO-criteria for unrestricted irrigation during both the summer and winter period respectively. During the summer period, the average total nitrogen was reduced more in ABPs (80%) than in DBPs (55%). Lower values were measured during the winter period. Seasonal nitrogen reductions of the two systems were significantly different ($P<0.05$). In DBPs, 33% and 15% of the total nitrogen was recovered into biomass and removed from the system via duckweed harvesting during the summer and winter period respectively. This study showed that there were differences in the environmental conditions and treatment efficiencies between the two systems.

Keywords: Algae ponds, duckweed ponds, faecal coliforms, *Lemna gibba*, treatment efficiency, wastewater

INTRODUCTION

Presently the application of conventional wastewater treatment systems in countries with a low GNP is limited because of high cost and technological complexity. World wide, there is a continuous interest for algae-based waste stabilisation pond systems that are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants. However, high algal concentrations of about 100 mg TSS/l may be occasionally reached in the effluent (Middlebrooks, 1995), causing severe clogging problems in advanced (drip) irrigation systems (Pearson *et al.*, 1995). These types of sustainable technologies for wastewater treatment, which are within the economical and technological capabilities of developing countries, need to be developed further. Introducing an aquatic plant (duckweed) to algae-based waste stabilisation pond in order to increase nutrient recovery in a so called duckweed-based pond could be an appropriate alternative.

Duckweed-based ponds (DBPs) are low cost and do not need sophisticated equipment, high energy or qualified labour input. Contrary to algae-based ponds (ABPs), DBPs may generate biomass that is known to be an excellent source of feed for fish or poultry raising (Skillicorn *et al.*, 1993; Oron, 1994) and yield good effluent quality for irrigation. Improvement of effluent quality will enhance the application of stabilisation pond systems and may offer important economic advantages for many developing countries. Different studies have shown that duckweed systems are capable of treating

wastewater (Edwards, 1980; Reddy and DeBusk, 1985; Zirschky and Reed, 1988; Alaerts *et al*., 1996) and suspended solids in the effluent are reported to be much lower than for conventional algae-based ponds.
Most of the studies available in literature comparing the performance of DBPs and ABPs were carried out on ponds with different configuration, loading rates and location. Therefore, the aim of this study is to assess, under identical conditions, the seasonal differences in process performance of the two systems.

MATERIALS AND METHODS

Experimental setup of the pilot plant

This study was carried out using a pilot-scale pond system at the new campus of Birzeit University (BZU), 26 km north of Jerusalem (31° 57′ 32″ N, 35° 10′ 43.8″ E, 750 above m.s.l). The pilot plant was built with reinforced concrete walls to ensure water tightness. It consists of a holding tank (2.2m length, 1.3m width and 1.9 m depth) followed by two parallel systems: algae-based ponds (ABPs) and duckweed-based ponds (DBPs). Each system consisted of a sequence of 4 equal ponds (3m length, 1m width and 0.9m depth) in series (Figure 1). Baffles at the outlet of each pond were constructed to avoid short-circuiting and transfer of floating materials to the consecutive ponds. The free board (30 cm) stopped the duckweed from being blown (or transported by water flow) to one end of the pond.

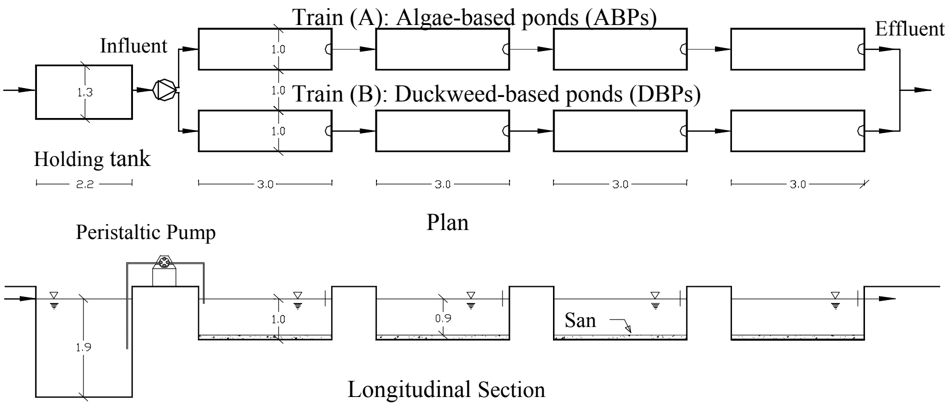


Figure 1. Schematic diagram of the pilot plant (All dimensions are in meters)

Pond operation and monitoring

Approximately 0.9 m^3 of wastewater from BZU was pumped daily to the holding tank from an aerated equalisation basin, which is part of the BZU activated sludge plant. BZU treatment plant receives its wastewater from the University new campus (3500 students) and septage (60 m^3/week) brought by tankers from the student dormitory two times a week. The experimental pilot plant system has been operated from December 1998 onwards as a continuous flow system. A peristaltic pump pumped the wastewater from the holding tank at equal rates (0.38 m^3/d to each system) to the ABPs and DBPs. DBPs were started with *Lemna gibba* species at a density of 600 g fresh weight m^{-2}.

Seasonal main characteristics of the influent wastewater to both pond systems are given in Table 1.
According to the classification by Metcalf and Eddy (1991) the wastewater is of weak to medium organic strength but contains medium to high nitrogen concentration. Wastewater composition is comparatively similar all year round. Further water transport to subsequent ponds in each train was by gravity. HRT of 7 days and water depth of 0.9 m is maintained in each pond. The final effluent of each system is flowing into a collection box and is channeled to the adjacent BZU activated sludge plant. A regular monitoring schedule was started 5 months after the pilot plant start-up. Grab samples (100-ml) were collected from the influent and the effluents of each pond once a week at 10:00 hours. For faecal coliforms (FC) analyses, samples were collected using sterile 100 ml glass bottles. Dissolved oxygen (DO) and pH were measured at 10 cm below the surface of the water column at 16:00 hours. DO and pH profiles over 24 hour periods were also established regularly.

Table 1. Mean seasonal physico-chemical and microbiological characteristics of the influent to the ABPs and DBPs. Number of measurements were 17, 4, 16 and 12 during summer, autumn, winter and spring respectively. Seasonal temperatures were calculated using the average daily ambient temperature that was gathered from the nearby weather station. Data are presented as means (± standard deviation). All values are in mg/l unless otherwise stated.

Parameter	Value			
	Summer	Autumn	Winter	Spring
Ambient T (^oC)	24 (3)	19 (1)	10 (3)	23 (4)
pH (-)	7.7 (0.2)	7.5 (0.2)	7.6 (0.2)	7.7 (0.2)
DO	0.8 (0.2)	1.1 (0.1)	0.4 (0.2)	0.9 (0.2)
BOD (total)	167 (3)	160 (27)	149 (20)	162 (17)
COD (total)	302 (56)	300 (42)	291 (38)	300 (26)
TSS	230 (66)	149 (30)	140 (24)	141 (44)
Chlorophyll *a* (µg/l)	71 (37)	42 (10)	8 (7)	10 (8)
Total-P	4.3 (0.3)	4.3 (0.1)	4.3 (0.3)	4.3 (0.3)
FC (CFU/100 ml)	2.24E+04	2.01E+04	1.95E+04	1.90E+04
NH_4^+-N	60 (6)	61 (4)	60 (6)	60 (3)
NO_3^--N	0.2 (0.2)	0.4 (0.1)	0.3 (0.2)	0.2 (0.1)
Organic-N	4 (4)	3.5 (1)	4 (3)	2.5 (1)

Analytical methods

The analytical methods were as described previously (Zimmo *et al.*, 2000).

Duckweed sampling and processing

During summer, autumn and spring seasons (the warm seasons) when duckweed growth was good, duckweed biomass was harvested every fifth day and duckweed density was restored to 600 g fresh weight m^{-2}. This density was selected to prevent overcrowding and to maintain sufficient cover to minimize the development of algae in duckweed ponds. Nitrogen content in duckweed was determined by analysing triplicate samples of stored and dried (105 ^{0}C) harvested duckweed from each pond three times per month. Fresh duckweed production rates were calculated from the final (D_f) and initial (D_i) fresh duckweed density during the harvesting cycle (t). The following formula was used: duckweed production rate = $(D_f-D_i)/t$. Dry weight of duckweed was calculated by drying sub-samples of the harvested duckweed at 105 ^{0}C.

RESULTS

Environmental conditions in the ponds

In the top 10-15-cm of the water column and during the afternoon, higher DO values were observed in ABPs (over-saturation during warm seasons and 5-6.4 mg/l during winter) than in DBPs (3.5-5.7 mg/l during warm seasons and 2.6-3.5 mg/l during winter). DO concentrations in both algae and duckweed ponds decreased rapidly with the distance from the water surface (Figure 2).

In all ponds of both systems, DO concentrations were approximately zero at the lower 30 cm of the water column. Differences observed in depths and between the two systems were significant ($P<0.05$).

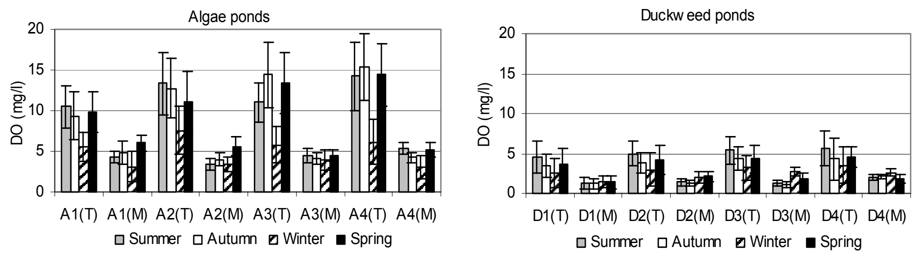


Figure 2. Seasonal means of DO concentrations at the top (T) and middle (M) depths during the afternoon in ABPs and DBPs. The error bars indicate the standard deviations.

The pH was highest near the surface of the water column and slightly decreased with the distance from the water surface. Higher pH values were observed in ABPs than DBPs (Figure 3). In warm seasons, the pH values of the water columns were 8.8-9.1 and 8.0-8.2 in ABPs and DBPs, respectively. Lower values were observed in winter (8.2-8.5 in ABPs and 7.6-7.9 in DBPs). In all ponds of the two systems, pH values in the sediment were approximately 7.5. Differences observed in depths and between the two systems were significant ($P<0.05$).

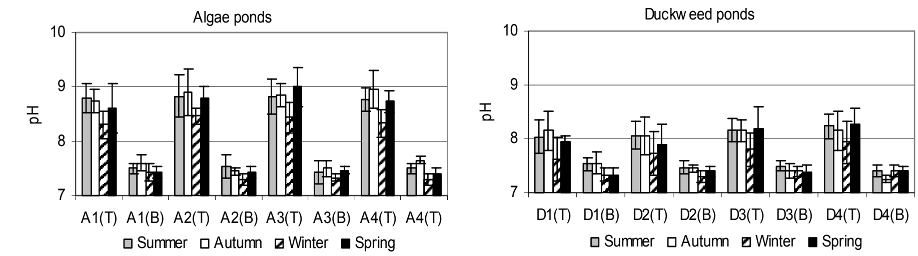


Figure 3. Seasonal means of pH values at the top (T) and bottom (B) depths in ABPs and DBPs. The error bars indicate the standard deviations.

Water temperature was highest at the surfaces than below in all ponds however, differences with depth were not significant (P>0.05). During warm seasons, shading caused by duckweed mat resulted in lowering the water temperature in DBPs by approximately 1ºC in comparison with water temperatures in ABPs. During the afternoon, temperatures were highest in all ponds. Differences between water and ambient temperature were not significantly different (P>0.05).
To determine the performance efficiency all year round as well as the effect of temperature, the average concentrations of different parameters from each pond of the two systems were calculated at four temperature ranges clustered as shown in Figure 4.

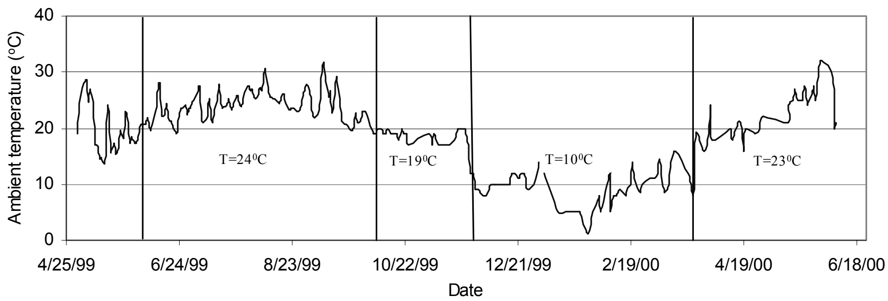


Figure 4. Average daily ambient temperature from the meteorological weather station at 600 m distance from the pilot plant.

Organic removal

The effluent of the ABPs contained higher concentrations of total BOD (Figure 5) and TSS (Figure 6) than DBPs. The influent organic load to each system during the year of monitoring ranged between 0.057 and 0.063 kg BOD/d.

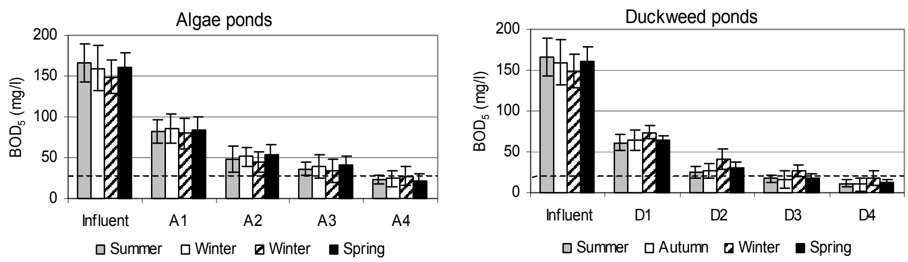


Figure 5. Seasonal means of BOD of influent and effluent of individual ponds of pilot plant. The error bars indicate the standard deviations. Dashed lines represent discharge standard.

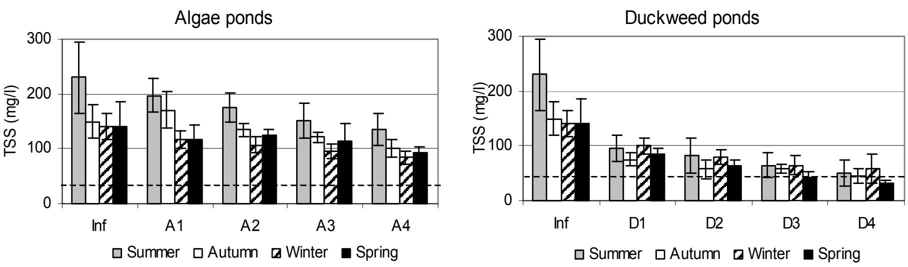


Figure 6. Seasonal means of TSS at ABPs and DBPs. The error bars indicate the standard deviations. Dashed lines present discharge standard.

Based on the daily organic load to the holding tank (≈ 0.2 kg BOD), each system will serve approximately two population equivalents. During warm seasons, the organic loading rate (202-211 kg ha^{-1} d^{-1}) to the first pond in both systems was approximately 50% lower than the maximum BOD loading rate for facultative ponds using the modified linear approximation of McGarry and Pescod's equation adjusted by Arthur (1983). During the cold period, the loading rate (189 kg ha^{-1} d^{-1}) was 26 % higher than the value calculated by the same model. In duckweed ponds, the annual average of the reductions in BOD (92%) and TSS (71%) were higher than that in algae-based ponds (BOD: 85% and TSS: 37%). In both systems, no significant reduction in removal of BOD and TSS were observed in the winter season. Chlorophyll *a* concentrations in ABPs (270-2390 µg/l) are approximately 6-15 times higher than concentrations in DBPs (42-157 µg/l). During the winter period chlorophyll *a* was less developed in ABPs in comparison with warm seasons. Microscopical investigations showed that *Euglena* was the dominant algae.

Faecal coliforms removal

Although duckweed-based ponds were producing lower concentrations of BOD and TSS, they appeared less efficient for faecal coliforms removal than the corresponding algae-based ponds. Seasonal faecal coliforms counts performed on the influent and pond effluents in both systems are shown in Table 2. Removal of FC was respectively 3.3-3.6 and 1.3-1.7 log units in ABPs and DBPs during the warm seasons and 2.0 and 0.7 during the cold season.

The first-order rate constants (based on the assumption of completely mixed conditions) of faecal coliforms die-off (K_b) were higher in ABPs than in DBPs and lower values in both systems were found during cold weather (Table 2). In algae based ponds, K_b ranges were between 0.7-2.0 and 0.3-0.6 day^{-1} during warm and cold seasons, respectively. In DBPs, these values were 0.16-0.45 and 0.09-0.14 day^{-1}.

Table 2. Mean seasonal values of FC (CFU/100 ml) of the influent and effluent of each pond in ABPs and DBPs.

	Summer	Autumn	Winter	Spring
Influent	2.24E+04	2.01E+04	1.95E+04	1.90E+04
ABPs				
A1	3.72E+03 (0.7)	2.95E+03 (0.8)	6.83E+03 (0.3)	3.07E+03 (0.7)
A2	3.50E+02 (1.4)	3.65E+02 (1.0)	1.98E+03 (0.4)	4.53E+02 (0.8)
A3	2.68E+01 (1.7)	3.00E+01 (1.6)	5.71E+02 (0.4)	4.30E+01 (1.4)
A4	2.00E+00 (2.0)	2.00E+00 (2.0)	1.08E+02 (0.6)	3.00E+00 (1.8)
DBPs				
D1	5.58E+03 (0.4)	7.75E+03 (0.2)	9.74E+03 (0.1)	6.24E+03 (0.2)
D2	1.82E+03 (0.3)	2.97E+03 (0.2)	6.11E+03 (0.1)	2.10E+03 (0.2)
D3	5.36E+02 (0.3)	7.35E+02 (0.4)	3.17E+03 (0.1)	5.43E+02 (0.2)
D4	2.56E+02 (0.2)	2.05E+02 (0.4)	1.80E+03 (0.1)	1.30E+02 (0.2)

Numbers in brackets represent the K_b values for first order removal, day^{-1}.

Nutrient removal

Phosphorus removal

The seasonal variations in total-P concentrations from each pond of the two systems are shown in Figure 7. Removal of total-P was respectively 74-79% and 74-92% in ABPs and DBPs during the warm seasons. Both systems had similar total-P removal efficiency (59% for ABPs and 61 for DBPs) during the winter season especially during the period when the duckweed cover disappeared due to low temperatures. The total-P reduction of the two systems were only significant ($p<0.05$) during the summer and spring seasons.

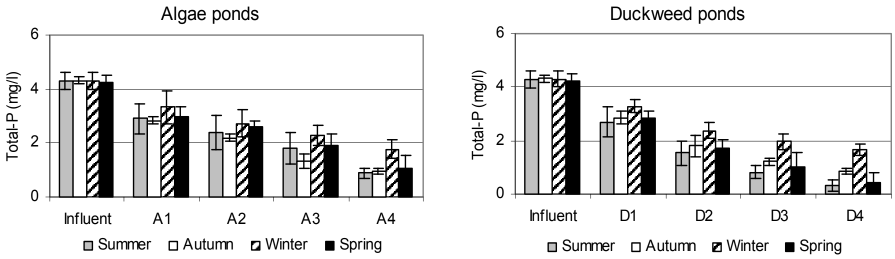


Figure 7. Seasonal means of total-P in ABPs and DBPs. The error bars indicate the standard deviations.

Nitrogen removal

Despite the lower BOD and TSS removal efficiency in ABPs, higher total nitrogen removal efficiency was achieved in comparison with DBPs (Figure 8).

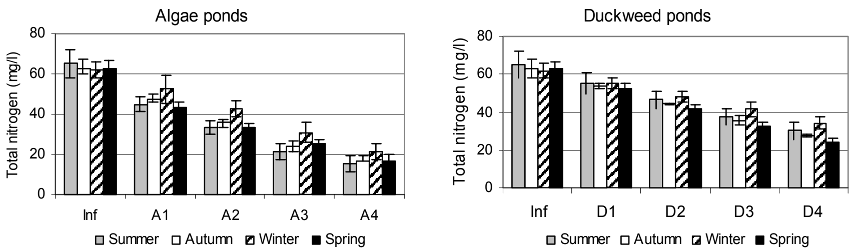


Figure 8. Seasonal means of total nitrogen at ABPs and DBPs. The error bars indicate the standard deviations.

Removal of nitrogen in ABPs was attributed to the nitrogen stored in the sediment, ammonia volatilisation and/or denitrification. In addition to the above mentioned removal processes, nitrogen recovery via duckweed harvesting is an important pathway for nitrogen removal in DBPs. The highest nitrogen removal in ABPs (77%) and DBPs (62%) was achieved during the summer and spring seasons respectively. For each individual pond of the two systems, nitrogen removal in the first pond of both systems was the highest. It was not surprising to measure lower nitrogen removal efficiency during the cold months (65% in ABPs system and 45% in DBPs) as microbial activities and removal mechanisms are expected to be reduced. Nitrogen removal of the two systems were significantly different ($P<0.05$) during the different seasons.

Total nitrogen in the influent was mainly composed of ammonium (NH_4^+-N) and only a small fraction (5%) of organic nitrogen. Throughout the treatment systems, higher values of organic nitrogen were measured in effluents of ABPs (5-10 mg/l during the warm seasons and 4-7 mg/l during the cold season) than DBPs (1-3 mg/l all year round). Despite the fact that part of the nitrogen in ABPs were incorporated in algal biomass as organic nitrogen and remained in the effluent, the overall nitrogen removal in ABPs was higher than DBPs. Annual nitrogen removal efficiencies in ABPs and DBPs were respectively, 73% and 54% after 28 days retention time.
Production of duckweed (Figure 9) in DBPs varied from 7.5 - 12.3 and 3 - 4 g dry weight m^{-2} d^{-1} during the warm and cold seasons respectively. Nitrogen content in duckweed was comparatively constant during various seasons. Average nitrogen content was 0.055±0.01 g N/g dry weight. The contribution of duckweed to the N-recovery as duckweed protein via duckweed harvesting was 33% and 11% of total nitrogen input to the system during the warm and cold seasons respectively. The annual nitrogen recovery represented 23% of total nitrogen input to the system.

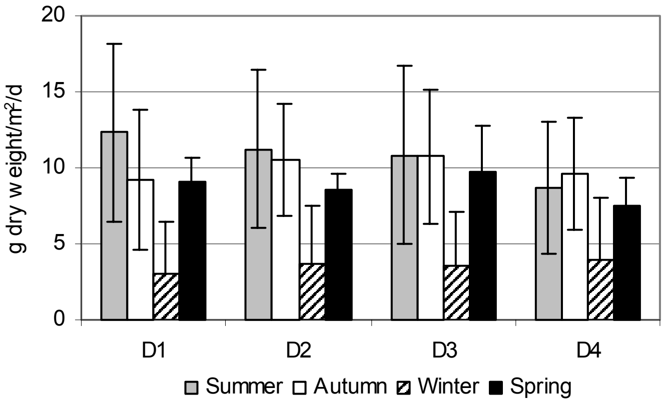


Figure 9. Seasonal means of duckweed growth rate at DBPs. The error bars indicate the standard deviation

DISCUSSION

Comparison of ABPs and DBPs revealed two obvious differences between the two systems. Firstly, ABPs usually develop high densities of algae in the water phase, whereas algae are almost absent in DBPs. Secondly, DBPs maintain a dense cover of duckweed on the surface, whereas ABPs do not have floating macrophytes on the water surface. The absence/presence of algae/duckweed resulted in differences in the environmental conditions in the two pond systems. The absence of algae in DBPs led to a reduction of DO levels in the water. There was low oxygen concentration produced from suspended algae, whereas the dense cover of duckweed may have reduced oxygen diffusion from the air into the water phase. Duckweed may supply some oxygen to the water via transport of atmospheric oxygen through the root zone (Moorhead and Reddy, 1988), but this contribution is probably small as compared to the oxygen production by algae. Higher diurnal pH fluctuation in ABPs due to algal photosynthetic activities was observed in comparison with DBPs. The diurnal variation in DBPs could be attributed to the photosynthetic activities by duckweed plant and/or algae that were present in low concentrations. The above differences in environmental conditions in the two systems have affected both chemical and biological activities involved in the treatment processes in the ponds.

Lower total BOD removal in ABPs was found due to the presence of suspended algae in the effluent. Algae development and high TSS concentrations in ABPs effluent will cause potential blockages of emitters if effluent is to be reused via drip irrigation. Also, effluent standard for discharging into wadis in most countries in the world (20 mg/l for BOD and 30 mg/l for TSS) could not be satisfied for the ABP system. In DBP system, effluent standards for BOD and TSS could be achieved after a HRT of 21 and 28 days respectively. Better removal of BOD and TSS in DBPs could be attributed to lower algal development and better sedimentation due to effect of shading and quiescent conditions provided by duckweed cover. BOD and TSS removal during the winter season were not reduced in both systems because of high HRT and lower organic loading rates especially in the last three ponds. BOD removal mechanisms in DBPs are not fully understood. It has been suggested that *Lemnaceae* can take up simple amino acids and other organic compounds from the water (Hillman, 1961; Landolt, 1986), but, Körner *et al*., (1998) concluded from laboratory studies with *Lemna gibba* that heterotrophic uptake of small organic compounds is not important. Nevertheless, they also found that COD removal was significantly faster in the presence of duckweed than in uncovered controls.

The results for BOD and TSS removal in DBPs were comparable to other studies using DBPs for wastewater treatment. Alaerts *et al*., (1996) reported a BOD removal efficiency of 95-99% in a full-scale treatment plant in Bangladesh at similar HRT of 20 days. Mandi (1994) reported BOD removal efficiency of 60-70% in a pilot plant, which was operated, with a cover of *Lemna gibba* at a HRT of about 7 days (equivalent to the effluent from the first duckweed pond in our system). These experiments were conducted with urban, domestic and industrial wastes with COD concentrations in the range of 305-530 mg COD/l and COD loading rate of 130-225 kg/ha.d. Also, Similar removal efficiency of 80% of TSS has been reported in DBP systems (Mandi, 1994; Bonomo *et al*., 1996; Zirschky and Reed, 1988).

Pathogen die-off result from complex interactions of several factors such as light radiation, depletion of nutrients, microbial antagonism, presence of antibacterial substances produced by algae, and high oxygen concentrations (Polprasert *et al.,* 1983; Pearson *et al*., 1987; Saqqar and Pescod, 1992). Curtis *et al*. (1992a, b) suggested that FC removal in waste stabilisation ponds depends on synergistic interaction between pH, dissolved oxygen, humic substances and light. Our study showed that introduction of duckweed into the pond affects these parameters. It seemed that direct sunlight, temporary and sharp rises in pH levels contributed to higher pathogen removal in ABPs than DBPs. In ABPs, Using the model of Marais (1974) for determining the faecal coliforms die-off coefficient K_b and using a temperature of 22 ^oC and 10 ^oC, which were the average ambient temperature during warm and cold seasons respectively, K_b values of 3.7 and 0.5 d^{-1} were obtained. The actual faecal coliforms in ABPs effluent were in agreement with predicted values derived using the first order equation of faecal coliforms reduction for determining effluent FC. FC levels as low as 2 CFU/100ml were detected in the effluent of the ABP system. Pond three and four presented the highest value of K_b however the effluent from the second (HRT=14 days) and third (HRT=21 days) algae pond already satisfied the WHO guideline for unrestricted irrigation (1989) during the warm and cold seasons respectively. This was not surprising since the influent concentration ($1.9x10^4$- $2.24x10^4$ per 100 ml) was lower than that of typical domestic wastewater. The K_b values for the DBPs were lower than for the ABPs (Table 2). Environmental conditions in the DBPs probably were not favorable for pathogen decay, due to reduced light penetration and algae growth. DBP system, however, was able to satisfy the WHO guideline at higher HRT (21 days during the summer and more than 28 days during winter) in comparison with ABP system.

The higher reduction of total-P in DBPs could be attributed to duckweed uptake and subsequent removal by harvesting. Total-P removal in ABPs was not effective due to the fact that part of the phosphorus were taken up by algae and remained in the pond effluents. Also, upon the death of algae biomass that settled to the bottom of the ABPs there would be a release of phosphorus in the water column.

Higher nitrogen removal was achieved in ABPs than DBPs. Removal via sedimentation during the summer period was presented elsewhere (Zimmo *et al*., 2000). The remaining removal could be attributed to denitrification in the sediment layer and/or ammonia volatilisation. Nitrogen removal in ABPs are comparable to those of Silva (1982) who obtained ammonium removal efficiency of 81% in a system of similar depth (1.0 m) and hydraulic retention time (29 days). However, Middlebrooks *et al*., (1982) reported higher removal values in systems with very long hydraulic retention times of 227 days. In addition to denitrification and/or ammonia volatilisation in DBPs, nitrogen recovery via duckweed harvesting (33% and 11% of total nitrogen input to the system during the warm and cold seasons respectively) presented an important removal mechanism. The nitrogen removal rate in the duckweed pond system was 1.2 and 0.9 g-N m^{-2} d^{-1} during the warm and cold seasons respectively. It was higher than values of 0.32 g-N m^{-2} d^{-1} reported by Alaerts *et al*., (1996) probably due to lower nitrogen concentration of the wastewater used in their system. van der Steen *et al*., (1998) reported higher values for surface nitrogen removal (1.7 g-N m^{-2} d^{-1}) in a shallow pond system that consisted of 7 duckweed ponds and 3 algal ponds.

Availability of macro-nutrients (N and P) in the first three duckweed ponds was in excess (65-24 mg-N/l) and seemed not to be a limiting factor for duckweed growth. Although nitrogen concentration in the fourth duckweed pond was still in excess (34-24 mg-N/l), longer root length characterized the duckweed plant. This probably could be due to depletion of phosphorus and micronutrients in pond water. Low growth rates were observed during the following times of the year: period of infestation with aphids (Zimmo *et al*., 2000), period of very high ambient temperature due to heat stress and winter period due to low temperature effect on duckweed growth. During the first two periods, duckweed turned yellowish, got much smaller in size and lacked the gibbous morphology. During the winter period, duckweed growth was dramatically reduced and duckweed cover totally disappeared when the water temperature was below 5 ^{0}C. The ammonium concentration in DBPs varied between 60 mg/l in the influent to the first pond to 20 mg/l in the effluent from the fourth pond, suggesting that ammonium concentration in this range did not affect the duckweed growth. Oron (1994) and Van der Steen *et al*., (1998) reported comparable values for nitrogen uptake rates. Other authors however reported lower uptake rates (470 mg N m^{-2} d^{-1}, Culley *et al*., 1978; 420 mg N m^{-2} d^{-1}, Corradi *et al*., 1981; 500 mg N m^{-2} d^{-1}, Tripathi *et al*., 1991) probably due to differences in the experimental conditions. It is not possible based on this research to determine the importance of the other nitrogen removal processes (ammonia volatilisation and denitrification) in ABP and DBP systems. This will be subject of further investigations. The wastewater used in this research is not representative for the region. Further work will be focussed to assess the relationship between the environmental condition and systems' performance when operated with high strength wastewater.

CONCLUSIONS

Environmental characteristics and removal efficiency of organic matter, nutrients and faecal coliforms in algae-based and duckweed-based stabilisation pond systems were studied during a period of 12 months. The higher values in pH and dissolved oxygen in ABPs were due to algal photosynthetic activities, which were suppressed in DBPs as a result of shading by the duckweed mat. The higher values in light penetration, pH and dissolved oxygen in algae ponds increase the removal of faecal coliforms compared to duckweed ponds. In ABPs, a HRT of 14 and 21 days during warm and cold seasons was required to achieve faecal coliforms reductions that comply with the WHO standard for unrestricted irrigation. In DBPs, these guidelines could be fulfilled at higher retention time (21 days during the warm seasons and more than 28 days during the cold season). Higher BOD and TSS removal efficiencies were achieved in DBPs compared to ABPs. In both systems, the removal of BOD and TSS did not differ significantly during the different seasons of warm and cold weather. Total-P was more effectively reduced in DBP system than in ABP system, irrespective of the season. Higher total nitrogen removal efficiencies can be achieved in algae system than in duckweed system despite the fact that approximately one third of the influent nitrogen to the DBPs is removed via duckweed harvesting. Lower removal efficiencies for nitrogen in both systems was obtained during the winter season. Nitrogen recovery via duckweed harvesting in DBPs was reduced substantially during winter season.

ACKNOWLEDGEMENTS

The authors are grateful to the Dutch government (SAIL) for financially supporting this research within the scope of the collaboration project between Birzeit University/West Bank and the International Institute for Infrastructural, Hydraulic and Environmental Engineering (IHE), The Netherlands. The authors like to thank the laboratory staff members of the MSc. Program at Birzeit for their technical support.

REFERENCES

Alaerts G.J., Mahbubar M.R., Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res.* **30**: 843-852.

Arthur J. P (1983). Notes on the design and operation of waste stabilisation ponds in warm climates of developing countries, World Bank Technical Paper No. 6, IBRD, Washington, DC.

Bonomo L., Pastorelli G., Zambon N. (1996). Advantages and limitations of duckweed-based wastewater treatment systems. *5th International Conference on Wetland System for Water Pollution Control*, VIII/2, 1-8, Vienna.

Corradi M., Copelli M. and Ghetti P. F. (1981). Colture di lemna su scarichi zootechnical. Inquinamento, **23**: 49-45.

Culley D. D., Gholson J. H., Chisholm T. S., Standifer L. C. and Epps E. A. (1978). Water quality renovation of animal waste lagoons utilizing aquatic plants. U.S. Environmental Protection Agency. Ada, Oklahoma. 166 pp.

Curtis T. P., Mara D. D. and Silva S. A. (1992a). The effects of sunlight on faecal coliforms in ponds: implications for research and design. *Wat. Sci. Tech.* **26**: 1729-1738.

Curtis T. P., Mara D. D. and Silva S. A. (1992b). Influence of pH, oxygen and humic substances on ability of sunlight to damage faecal coliforms in waste stabilisation pond water. *Appl.Environ.Microbiol*. **58**: 1335-1343.

Edwards P. (1980). Food potential of aquatic macrophytes. ICLARM Studies and Reviews 5, 51 pp.

Hillman S. (1961). The *Lemnaceae*, or duckweed, a review of descriptive and experimental literature. *Botanical Reviews*. **27**: 221-287.

Körner S., Lyatuu G. B., Vermaat J. E. (1998). The influence of *Lemna gibba L.* on the degradation of organic material in duckweed-covered domestic wastewater. *Wat. Res.* **32**: 3092-3098.

Landolt E. (1986). The family of *Lemnaceae* - a monographic study. *Veröffentlichungen des Geobotanischen Institutes der ETH Zürich*, Band **71**: 566 pp.

Mandi L. (1994). Marrakesh wastewater purification experiment using vascular aquatic plants *Eichhornia crassipes* and *Lemna gibba. Wat. Sci. Tech.* **29**: 283-287.

Marais G. v. R (1974). Faecal bacterial kinetics in stabilisation ponds. *J. Environ.Eng. ASCE*. **100**: 119-139.

Metcalf and Eddy (1991). Wastewater engineering. Treatment, disposal and reuse. Tchobanoglous G. and Burton F. L. [eds.], McGraw – Hill. New York.

Middlebrooks E. J., Middlebrooks C.H., Reynolds J. H., Watters G. Z., Reed S.C. and George D.B. (1982). Wastewater stabilisation lagoon design, performance and upgrading. Macmillan, NY, USA.

Middlebrooks E. J. (1995). Upgrading pond effluents: an overview. *Wat. Sci. Tech.* **31**: 353-368.

Moorhead K.K. and Reddy K.R. (1988). Oxygen transport through selected aquatic macrophytes. *J. Environ. Qual.* **17**: 138-142.

Oron G. (1994). Duckweed culture for wastewater renovation and biomass production. *Agric. Wat. Manag.* **26**: 27-40.

Pearson H.W., Mara D.D., Mills S.W. and Smallman D.J. (1987). Physico-chemical parameters influencing faecal bacterial survival in waste stabilization ponds. *Wat. Sci. Tech.* **19**: 145-152.

Pearson H.W., Mara D.D. and Arridge H.A. (1995). The influence of pond geometry and configuration on facultative and maturation waste stabilisation pond performance and efficiency. *Wat. Sci. Tech.* **31**: 129-139.

Polprasert C., Dissanayake M.G. and Thanh N.C. (1983). Bacterial die-off kinetics in waste stabilization ponds. *J. WPCF.* **55**: 285-296.

Reddy K. R., DeBusk W. F. (1985). Nutrient removal potential of selected aquatic macrophytes. *J. Environ. Qual.* **14**: 459-462.

Saqqar M. M. and Pescod M. B. (1992). Modelling coliform reduction in wastewater stabilization ponds. *Wat. Sci. Tech.* **26**: 1667-1677.

Silva S. A. (1982). On the treatment of domestic sewage in waste stabilisation ponds in N.E. Brazil. PhD thesis, University of Dundee, UK.

Skillicorn P., Spira W., Jouney W. (1993), Duckweed Aquaculture, a new aquatic farming system for developing countries. The World Bank, Washington, USA.

Tripathi B. D., Srivastava J. and Misra K. (1991). Nitrogen and phosphorus removal capacity of four chosen aquatic macrophytes in tropical freshwater ponds. *J. Environ. Qual.* **18**: 143-147.

Van der Steen P., Brenner A. and Oron G. (1998). An integrated duckweed and algae pond system for nitrogen removal and renovation. *Wat. Sci. Tech.* **38**: 335-343.

WHO (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. *Technical report series* 778, 74 pp.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2000). Nitrogen transformation and treatment efficiency in algae-based and duckweed-based stabilisation ponds. Conference proceeding "*Managing Water and Waste in the New Millennium*"

Zirschky J. and Reed S. C. (1988). The use of duckweed for wastewater treatment. *J. WPCF.* **60**: 1253-1258.

Chapter 5

EFFECT OF ORGANIC SURFACE LOAD ON PROCESS PERFORMANCE OF PILOT SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

Submitted as:

Zimmo O. R., van der Steen N. P. and Gijzen H. J. effect of organic surface load on process performance of pilot scale algae and duckweed-based waste stabilisation ponds.
Journal of Environmental Engineering, ASCE.

EFFECT OF ORGANIC SURFACE LOAD ON PROCESS PERFORMANCE OF PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

ABSTRACT

Removal efficiencies in pilot-scale algae-based ponds (ABPs) and duckweed-based ponds (DBPs) were assessed during 2 periods of 4 months each. During period 1 and 2, the effect of low and high organic loading was studied. A linear correlation between ponds organic surface loading rates and the corresponding BOD removal rates was observed in both systems. For both periods, higher BOD and TSS removal efficiencies were found in DBPs compared to ABPs. Nitrogen removal rates (λ_r) in ABPs were linearly correlated with BOD surface loading rates ($\lambda_{s,BOD}$) and nitrogen loading rates ($\lambda_{s,N}$), while in DBPs, N-removal rates were almost constant irrespective of $\lambda_{s,BOD}$ or $\lambda_{s,N}$. Overall N-removal rate in the algae system was significantly higher than that in duckweed system. Organic loading had no effect on total phosphorus removal efficiency in both systems. Higher P-removal efficiency was achieved in the duckweed system than in the algae system. In ABPs as well as DBPs, faecal coliforms were better removed during low organic loading in comparison with high organic loading. During the two operational periods, higher faecal coliform removal efficiency in the algae system than in the duckweed system was observed.

Keywords: algae, duckweed, organic load, performance, stabilisation ponds, wastewater.

INTRODUCTION

Wastewater characteristics vary from place to place depending on several factors such as water usage pattern, nutritional habits and wastewater collection system (separate versus combined sewers). Strength of wastewater can highly affect the various processes (biological, physical and chemical) responsible for pollutant removal in wastewater treatment technologies. Mara (1976) suggested that the choice of wastewater treatment should be compatible with local habits and social and religious practice. Waste stabilisation ponds are in this paper referred to as algae-based ponds, are low cost (provided land is available at a reasonable cost) and easy to operate wastewater treatment systems. The functions of three types of ponds classified in ABPs: anaerobic, facultative and maturation are determined mainly by the organic load. The use of duckweed in wastewater treatment is not well documented, nor are design criteria available. The experiences reported so far, however, suggest that this technology shows great promise for low cost wastewater treatment and nutrients recovery. Duckweed-based systems may be used for secondary treatment (Ngo, 1987). The performance of duckweed ponds for wastewater treatment in relation to wastewater strength has not been evaluated before, although general monitoring studies are reported (Alaerts *et al*., 1996; Al-Nozaily *et al*., 2000; Edwards, 1992; Reddy and DeBusk, 1985; Zirschky and Reed, 1988). Nutrient recovery in duckweed ponds is well-documented (Gijzen, 2001). Suspended solids in duckweed pond effluent are reported to be much lower than for conventional algae ponds (Zimmo *et al*., 2002).
Using duckweed for wastewater treatment was recommended in literature for treating wastewater of low organic strength. Mandi (1995) demonstrated that *Lemna gibba*

could be used in primary treatment of wastewater with COD ranging from 305-530 mg l^{-1} but lacks the ability to grow on undiluted domestic wastewater or industrial wastewater (COD =1389 and 1667 mg l^{-1}, respectively). According to Copelli *et al*. (1982), the maximum COD in water tolerated by duckweed ranges from 300 to 500 mg l^{-1}. Al-Nozaily *et al*. (2000) found in a batch experiment at initial organic surface loading higher than 800 kg filtered COD/ha that oxygen supply might become a limiting factor for COD removal. Other factors related to wastewater strength like ammonia toxicity are reported in literature to have toxic effects on duckweed (Caicedo *et al*., 2000; Clement and Merlin, 1995; Wang, 1991). In a comparative study between algae and duckweed-based ponds treating wastewater of low strength (Zimmo *et al*., 2002), DBPs were found to be more efficient in removal of organic matter (BOD and TSS) than ABPs. However, the faecal coliform reduction was higher in ABPs due to solar radiation related harsh environmental conditions that enhance decay of faecal coliforms.
The aim of this study is to compare the performance of algae and duckweed-based ponds in relation to wastewater strength.

MATERIALS AND METHODS

Experimental setup of the pilot plant

The effect of organic load on removal efficiency of algae-based ponds (ABPs) and duckweed-based ponds (DBPs) was investigated in a pilot-scale pond system at the new campus of Birzeit University (BZU), 26 km north of Jerusalem (31° 57′ 32″ N, 35° 10′ 43.8″ E, 750 above m.s.l). The pilot plant was built with reinforced concrete walls to ensure water tightness. Each system consisted of 4 equally sized ponds in series (3m length, 1m width and 0.9m depth) (Figure 1) and was fed with a constant flow rate of 0.38 m^3 d^{-1}. The theoretical retention time in each pond was 7 days. Baffles at the outlet of each pond were constructed to avoid short-circuiting and transfer of floating materials to the consecutive ponds.

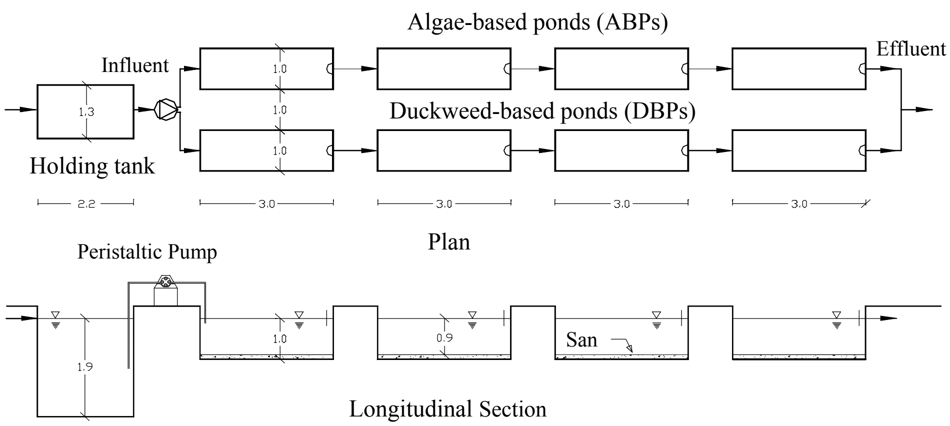


Figure 1. Schematic diagram of the pilot plant (All dimensions are in meters).

ABPs and DBPs were fed with three categories of influents during three successive periods. From December 1999 to August 2000, treatment systems received wastewater from the Birzeit University New Campus of low organic strength according to Metcalf

& Eddy (1991). From September 2000 to February 2001 wastewater from Al-Bireh was used which contained high nitrogen concentrations (>100 mg/l). This resulted in complete decay of duckweed and the ponds were operated temporarily as algae-based ponds. From February to August 2001 treatment systems received strong wastewater from the Faculty of Commerce. Two periods of four months each were monitored in order to compare the removal efficiency of the two systems at low and high organic loading conditions. The first period, when the two systems were fed at low organic loading rate, was from April to August 2000. The second period, when the two systems were fed with wastewater of high organic strength according to Metcalf & Eddy (1991), was from April to August 2001. Physico-chemical and microbiological characteristics of the influent wastewater during the two experimental periods are shown in Table 1. Values are averages of 16 measurements. Each system serves approximately 2 and 4 population equivalents during period 1 and period 2, respectively. During the course of the two experimental periods, water samples were collected from the influent and effluents of each pond. Removal of COD, ammonia, total-N, total-P (P), N-recovery in the DBPs and faecal coliforms removal were investigated. Dissolved oxygen (DO) and pH at the top, in the middle and just above the sediment were measured at 14:00 hours. Ambient temperatures were calculated using the average daily temperature that was gathered from the nearby weather station.

Table 1. Mean physico-chemical and microbiological characteristics (± standard deviation) of the low and high strength wastewater. Data are presented as means.

Parameter	Low strength wastewater	High strength wastewater
Ambient T (^oC)	24 (3)	25 (4)
pH (-)	7.7 (0.2)	7.6 (0.2)
BOD (mg l^{-1})	167 (15)	375 (32)
TSS (mg l^{-1})	149 (45)	189 (29)
Total-P (mg-P l^{-1})	4.3 (0.13)	4.3 (0.2)
FC (CFU/100 ml)	1.98E+04 (2.10E+03)	1.35E+05 (3.68E+03)
Total-N (mg-N l^{-1})	61.7 (2.8)	69.0 (3.2)
Organic-N (mg-N l^{-1})	4.0 (3.4)	5.8 (2.6)

Analytical methods

The analytical methods for nitrogen compounds (Kjeldahl, ammonium nitrite and nitrate), BOD, TSS, Total-P, FC, pH and temperature were as described previously (Zimmo *et al.*, 2000).

Duckweed sampling and processing

The duckweed (*Lemna gibba*) biomass in DBPs was maintained at a density of 600 g fresh weight m^{-2} by harvesting the surplus every five days. This density was selected to prevent overcrowding and to maintain sufficient cover to minimize the development of algae in duckweed ponds. N and P content of duckweed was determined by analysing triplicate samples of stored and dried (105 ^0C) harvested duckweed from each pond three times per month. Fresh duckweed production rates were calculated from the final (D_f) and initial (D_i) fresh duckweed density during the harvesting cycle (t). The following formula was used: duckweed production rate = $(D_f-D_i)/t$. Dry weight of duckweed was calculated by drying sub-samples of the harvested duckweed at 105 ^oC.

RESULTS

In both ABPs and DBPs lower dissolved oxygen (DO) values were found in the top and middle layer during period 2 in comparison with period 1. In period 1, Higher DO concentrations were measured in the surface layer of ABPs (10-14 mg l^{-1}) than in DBPs (4-6 mg l^{-1}). DO levels in the surface layer in the second period were 5-7 mg l^{-1} and 4-5 mg l^{-1}, respectively for the ABPs and DBPs. DO concentration in all ABPs and DBPs during the two experimental periods decreased from the top to the bottom. Anaerobic conditions were found just above the sediments. Significantly higher pH values were measured in ABPs during the two periods of operation (8.6-9 and 8.3-8.9) compared to DBPs (8-8.2 and 8-8.3). pH was slightly higher near the surface in both experimental periods.

Total BOD and TSS concentrations in ABPs and DBPs during the two experimental periods are shown in Figure 2 and Figure 3, respectively. Average reduction in BOD (period 1: 93%; period 2: 91%) in DBPs was higher than in ABPs (86 and 85%). Similarly TSS removal efficiency was 81% during the two periods in the DBPs and only 47 and 59% during period 1 and 2 in the ABPs, respectively. In both systems, higher BOD and TSS removal rates were achieved during period 2 as compared to period 1.

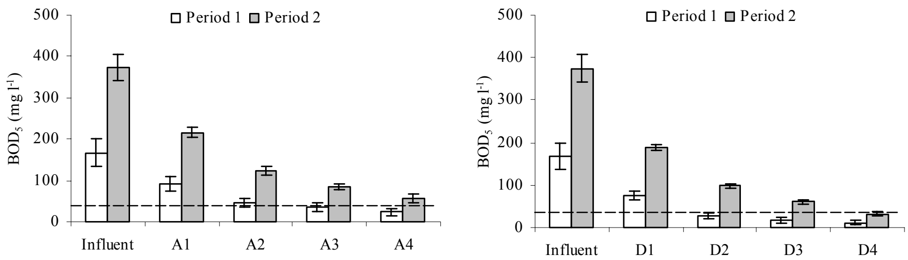
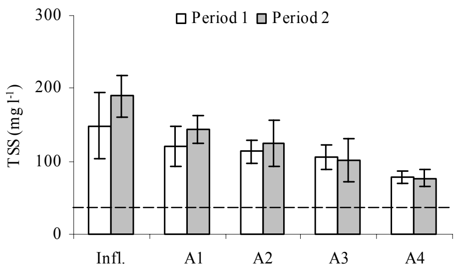


Figure 2. Means of BOD of influent and effluent of individual ponds during period 1 and period 2. The error bars indicate the standard deviations (n=16). Dashed lines present discharge standard (20 mg l^{-1}).

The organic loading rate in the first pond during period 2 (468 ± 56 kg $ha^{-1}d^{-1}$) was approximately twice as high as during period 1 (167±9 kg $ha^{-1}d^{-1}$). This low loading rate was similar to the values recommended by Arthur (1983) for facultative ponds, taking 23 ^{o}C as design temperature. Facultative conditions prevailed in the first pond of both systems and high BOD removal efficiency (46-50%) was achieved in both the first algae and first duckweed pond with no significant difference. Significantly higher removal of BOD was found in the successive three DBPs in comparison with ABPs during both periods.



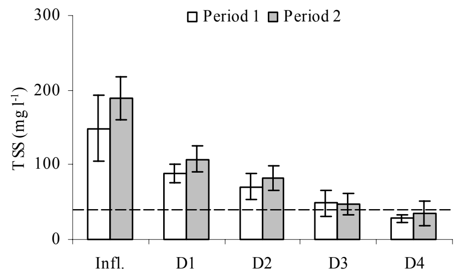


Figure 3. Means of TSS of influent and effluent of individual ponds during period 1 and period 2. The error bars indicate the standard deviations (n=16). Dashed lines represent discharge standard (30 mg l^{-1}).

Table 2 shows the total-N concentration and percentage removal in ABPs and DBPs during period 1 and period 2. In ABPs similar N-removal efficiency during period 1 (74%) and period 2 (68%) was found. Also, DBPs behaved similarly during period 1 (62%) and period 2 (59%). N-removal rate in ABPs was significantly higher (oneway ANOVA, $P<0.05$) than that in DBPs during the two experimental periods. Higher values of organic nitrogen (data not shown) were measured in the effluents of ABPs (5-10 mg l^{-1}) than DBPs (1-3 mg l^{-1}) due to presence of algae biomass in the former system.

Table 2. Total-N concentration, cumulative percentage of N-removal and pond surface N-removal rates of the ABPs and DBPs during the two experimental periods. Numbers in brackets represent standard deviation (n=16).

	Total-N concentration mg-N l^{-1}		Cumulative N-removal %		Removal rate (mg-N m^{-2} d^{-1})	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Inf	62 (2.8)	69 (3.2)				
A1	51 (2.4)	56 (3.6)	25%	21%	2022 (322)	1906 (349)
A2	40 (1.7)	43 (2.2)	44%	38%	1454 (305)	1458 (328)
A3	26 (2.9)	32 (1.7)	58%	53%	1138 (284)	1367 (132)
A4	17 (3.3)	21 (1.0)	74%	68%	1226 (209)	1338 (156)
D1	51 (3.7)	58 (2.8)	15%	14%	1165 (370)	1281 (253)
D2	42 (2.5)	48 (2.6)	32%	30%	1357 (322)	1356 (244)
D3	33 (2.2)	39 (3.3)	48%	45%	1315 (325)	1356 (225)
D4	23 (2.1)	30 (3.8)	62%	59%	1092 (288)	1257 (169)

Production of duckweed during period 1 in the four duckweed ponds was 7.7, 8.8, 10.0 and 12.3 g dry weight m^{-2} d^{-1}, respectively. Corresponding values during period 2 were 2.5, 5.0, 10.1 and 10.3 g dry weight m^{-2} d^{-1}, respectively. A decrease in duckweed production during this period was observed in the first and second ponds. Increase or decrease of N-uptake by duckweed was due to increase or decrease in duckweed production rather than change in duckweed N-content, which was found to be constant around 0.055 ± 0.01 g N/g dry weight (n = 15). Average duckweed uptake rates of N during the two experimental periods are shown in Table 3. N-removal by duckweed harvesting was 30% and 19% of total N-input to the system during period 1 and period 2, respectively.

Table 3. N-uptake rates by duckweed in DBPs during period 1 and period 2. Data are presented as means (± standard deviation).

	N-uptake rates by duckweed (mg-N $m^{-2}d^{-1}$)			
	D1	D2	D3	D4
Period 1	461 (125)	530 (91)	602 (122)	593 (136)
Period 2	122 (60)	283 (151)	550 (125)	549 (124)

Total-P, removal percentage and removal rates in ABPs and DBPs during the two experimental periods are shown in Table 4. In both periods 1 and 2, P-removal rates were higher in DBPs than in ABPs. In the ABPs average P-removal rates were 93±40 and 92±43 mg-P $m^{-2}d^{-1}$ during period 1 and period 2, respectively. While significantly higher average removal rates were achieved in the DBPs system (121±31 in period 1 and 125±30 in period 2). The highest removal rate in an individual pond occurred in the first pond of the two systems. P-removal in both periods for the last three algae ponds was similar, ranging from 68 to 91 mg-P $m^{-1}d^{-1}$. P-removal in both periods for the last three DBPs was not significantly different from each other.

Table 4. Total-P, cumulative percentage of P-removal and pond surface P-removal rates of the ABPs and DBPs during period 1 and period 2. Numbers in brackets represents standard deviation (n=16).

	Total-P mg-P l^{-1}		Cumulative P-removal %age		P-removal rate mg-P m^{-2} d^{-1}	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
Inf	4.3 (0.2)	4.3 (0.2)				
A1	3.2 (0.3)	3.1 (0.2)	24%	28%	131 (44)	154 (25)
A2	2.7 (0.1)	2.6 (0.2)	37%	40%	71 (39)	68 (28)
A3	2.0 (0.2)	2.0 (0.2)	53%	53%	91 (19)	69 (22)
A4	1.4 (0.2)	1.4 (0.1)	68%	67%	78 (29)	76 (25)
D1	3.1 (0.3)	3.1 (0.2)	27%	28%	149 (36)	154 (28)
D2	2.3 (0.1)	2.2 (0.1)	47%	50%	108 (30)	120 (24)
D3	1.3 (0.1)	1.3 (0.1)	69%	71%	122 (12)	115 (25)
D4	0.5 (0.1)	0.4 (0.1)	88%	91%	105 (21)	110 (26)

Average P-uptake by duckweed (Table 5) during period 1 (74 ± 20 mg-P $m^{-2}d^{-1}$) was higher than that during period 2 (46 ± 30 mg-P $m^{-2}d^{-1}$). Comparing the P-uptake rates by duckweed during the two experimental periods, a decrease by 73% and 43% in pond 1 and pond 2 respectively was observed during period 2. The contribution of P-uptake by duckweed was 60% and 38% of total P-input to the system during period 1 and period 2, respectively.

Table 5. P-uptake rates by duckweed in DBPs during period 1 and period 2. Data are presented as means (± standard deviation).

	P-uptake rates (mg-P $m^{-2}d^{-1}$)			
	D1	D2	D3	D4
Period 1	64.9 (16.2)	75.9 (18.3)	76.8 (24.1)	78.4 (17.9)
Period 2	16.5 (18.6)	33.2 (24.2)	67.9 (18.8)	67.3 (18.6)

Removal of FC in ABPs during period 1 and 2 were 3.8 and 3.4 log units, respectively (Table 6). Lower FC removal were observed in DBPs (period 1: 2.2 log units; period 2: 1.8 log units) (Table 6). FC removal during period 1 was significantly higher than removal during period 2 in the first pond in both ABPs system and DBPs systems. In both periods 1 and 2, the first-order rate constant (based on the assumption of completely mixed conditions) of faecal coliform die-off (K_b) was higher in ABPs than DBPs (Table 6). In ABPs, K_b ranged between 0.69-1.79 and 0.17-1.66 day^{-1} during period 1 and period 2 respectively. These values in DBPs were 0.26-0.45 and 0.12-0.34 day^{-1}.

Table 6. Mean values of FC (CFU/100 ml) of influent and effluent of each pond in ABP and DBP systems. Numbers in brackets represent the K_b values for first order removal day^{-1}.

		ABPs				DBPs			
	Inf.	A1	A2	A3	A4	D1	D2	D3	D4
Period 1	$1.98x10^4$	$3.41x10^3$ (0.69)	$4.23x10^2$ (1.01)	$4.26x10^1$ (1.28)	$3.14x10^0$ (1.79)	$5.08x10^3$ (0.41)	$1.82x10^3$ (0.26)	$4.99x10^2$ (0.38)	$1.20x10^2$ 2 (0.45)
Period 2	$1.35x10^5$	$6.23x10^4$ (0.17)	$6.64x10^3$ (1.2)	$6.44x10^2$ (1.33)	$5.10x10^1$ (1.66)	$7.36x0^4$ (0.12)	$2.53x10^4$ (0.27)	$7.86x10^3$ 3 (0.32)	$2.35x10^3$ (0.34)

DISCUSSION

Environmental conditions and BOD removal

The lower DO values observed in DBPs compared to ABPs may be the result of reduced diffusion of oxygen from the air into the water column and of reduced photosynthetic production of oxygen by phytoplankton in the water column because of poor light penetration. However, even in DBPs the top and middle layer of the water column always remained aerobic despite the high organic loading rate in the first ponds (468 kg ha^{-1} d^{-1}) during period 2. Enough oxygen was present for heterotrophic metabolism. Like for ABPs, BOD removal in DBPs could be explained by aerobic (top 70 cm of the water column) and anaerobic degradation (lower 20 cm of the water column) of organic matter. The biomass attached to duckweed is expected to play a minor role in BOD removal due to the intensive duckweed harvesting practice (every 5 days) and the small surface area to pond volume ratio. On the other hand, the presence of the duckweed mat resulted in higher removal of BOD in DBPs than ABPs despite the higher DO in the latter system. Better removal of BOD and TSS in DBPs could be attributed to the reduced algae development (Zimmo *et al.*, 2002). Apparently the rate of algae development in ABPs exceeded that of sedimentation and this resulted in an increase in effluent organic matter.

The removal rates (λr) of BOD in ABPs and DBPs were found to be linearly related to pond influent BOD surface loading (λs) (Figure 4). Combining the values calculated for all ponds in each system during the two experimental periods yielded the following equations: $\lambda r = 0.44\ \lambda s - 6.7$ and $\lambda r = 0.51\ \lambda s - 1.73$ for ABPs and DBPs, respectively. Correlation coefficients were >0.97. This suggests a causal relation between loading rates and the BOD removal rates. Therefore, the increase in organic loading in the two systems significantly improves pond performance organic removal rates. The lower organic removal rates in both systems at later stage of treatment could be explained by the fact that the remaining organic matter at this stage of treatment would be less biodegradable and result in lower removal rates.

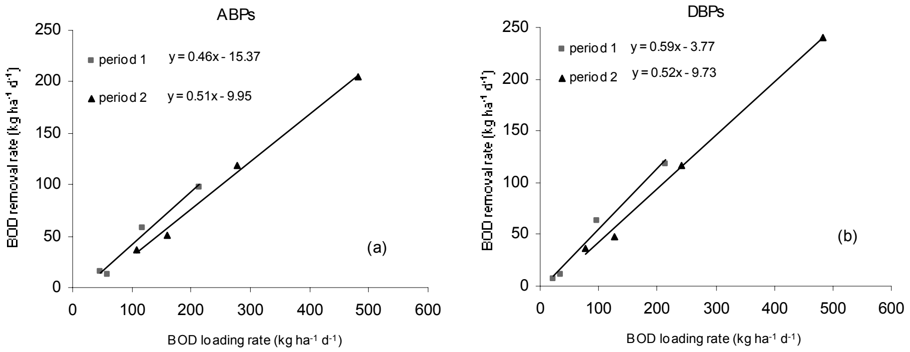


Figure 4. Mean pond BOD removal rate as a function of BOD surface loading rate in the four algae ponds (a) and in the four duckweed ponds (b) during period 1 and period 2.

N-removal

N-removal rates in ABPs seem to correlate with BOD loading rates (R^2=0.65) and with N-loading rates (R^2=0.80), while in DBPs N-removal rates were relatively constant irrespective of BOD loading rates or N-loading rates (Figure 5 a,b).

Duckweed growth was significantly less during period 2, during which the organic surface load was higher but the nitrogen load equal to period 1. During both periods free ammonia toxicity probably did not play a major role. During Period 1, the well-maintained duckweed cover prevented elevation of pH through algae growth due to the shading provided by the duckweed cover. Therefore the unionised fraction of ammonia was kept below the level of toxicity reported by Caicedo *et al.* (2000), Clement and Merlin (1995) and Wang (1991). During period 2, the ammonia concentration as a function of the pH and ammonium concentration in pond water was calculated using the equation by Clement and Merlin (1995) and found to be <3 mg-N l^{-1} and therefore not toxic. This suggests that the reduction in duckweed production in the second period in these two ponds was not due to ammonia toxicity but rather due to high organic loading. The development of a slime layer on duckweed roots in pond 1 and pond 2 during the period of high organic loading was observed which probably inhibited plant nutrient uptake. N-uptake rates by duckweed are higher than reported values in a full-scale duckweed lagoon in Bangladesh (2.6 kg-N $ha^{-1}d^{-1}$) (Alaerts *et al.*, 1996) and pilot plant sewage lagoon in Yemen (2.2-4.4 kg-N $ha^{-1}d^{-1}$) (Al-Nozaily, 2001).

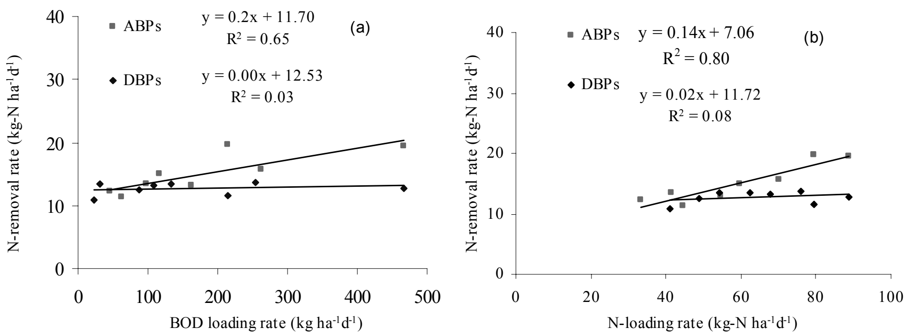


Figure 5. Pond N-removal rate as a function of influent BOD loading rate (a) and influent N-loading rate (b) during period 1 and period 2 for algae and duckweed-based ponds.

N-uptake by duckweed in DBPs system decreased from 30% of total-N input to the system during period 1 to 19% during period 2. However, this had no noticeable effect on overall N-removal. The decrease in N-uptake by duckweed during period 2 in combination with the absence of change in the overall N-removal suggests that an increase in N-removal due to nitrification-denitrification, ammonia volatilisation and sedimentation of particulate nitrogen (Total nitrogen removal – nitrogen uptake via duckweed). The importance of different N-removal mechanisms at low and high organic loading in algae and duckweed-based stabilisation ponds will be the subject of further research.

Total-P removal

The increased P-removal rates in the first pond of the two systems compared with successive ponds were due to settling of phosphorus associated with solids present in the influent. In ABPs, P-removal is due to sedimentation of incorporated phosphorus in particulate and decayed algae and due to biological phosphorus removal. In addition to the previous mechanisms, duckweed uptake of phosphorus in the DBPs and subsequent harvesting accounted for higher removal compared to ABPs. P-removal rates in DBPs are comparable with values obtained by Al-Nozaily *et al.* (2000) in laboratory scale experiments with domestic sewage.

The P-removal efficiency (67-68%) in DBPs was not significantly different during the two experimental periods (Table 5). During period 2, P-uptake by duckweed decreased by 73% and 43% in the first and second duckweed pond, respectively. The reduction in P-uptake by duckweed was due to the effect of organic loading on duckweed growth as discussed earlier. Except for pond 1 and pond 2 during period 2, P-uptake rates were comparable to data reported by Culley *et al.* (1978), Reddy and DeBusk (1985) and Zirschky and Reed (1988). The phosphorus removal rates via other mechanisms rather than uptake by duckweed were 50 and 77 mg-P $m^{-2}d^{-1}$ during period 1 and period 2, respectively. This removal significantly increased with increasing organic loading rate despite the decrease of P-uptake by duckweed during this period. The algae P-uptake (as a result of incomplete duckweed cover in the first two ponds), which partly settled in the sediment could explain the increase during this period.

FC-removal

The removal of faecal coliforms in the algae system and in the duckweed system was significantly higher during period 1 than during period 2. The reduction in FC removal during period 2 in both systems was mainly due to the lower removal rate occurring in the first pond. In the algae system, it was found that high concentration of algae matter as indicated by measurement of chlorophyll a (data not shown) during period 2 (while the pH remained similar, data not shown), may have a negative effect on FC decay due to light attenuation (van der Steen *et al*., 2000). It is not possible from this research to clearly define which mechanism is responsible for the higher removal in the first pond of the two systems during period 1 compared with rates in period 2. FC removal in DBPs was significantly lower than in the ABPs during the two experimental periods. In a tracer study conducted earlier in the ABPs and DBPs and reported elsewhere (Zimmo *et al*., Submitted), similar hydraulic behaviors in the systems were found. This suggests that other factors rather than hydraulic behaviors are responsible for differences in pathogen removal of both systems. The absence of algae photosynthesis in DBPs prevented the typical increase of DO and pH values observed in the ABPs. The photo-oxidation, high DO and high pH triggered FC-decay will hardly occur in DBPs (Curtis *et al*., 1992a,b).

Consequences for pond design

If N-concentration in the effluent is reduced to 15 mg l^{-1} and when applying such effluents at an irrigation rate of about 2 m y^{-1}, this results in N-dosage of 300 kg $ha^{-1}y^{-1}$. This practice could significantly reduce or even eliminate the use of commercial fertiliser. Based on influent total-N concentrations of 65mg-N l^{-1}, typically found in high strength domestic wastewater and if the aim of treatment is to bring N-concentration to 15 mg-N l^{-1}, DBPs would require 10% more surface area than ABPs. If constructed wetlands are to be used to obtain the same level of N-removal, the area required for treatment calculated by the model proposed by Hammer and Knight (1994) is 1.5-2.0 times the area needed for DBPs. It can be concluded that ABPs are the most efficient natural systems for N-removal, but the algal biomass in the system effluent cannot be easily harvested and consequently nutrients are released again in the environment upon degradation. To reduce phosphorus concentration to 3 mg-P l^{-1}, calculated to be enough to substitute chemical fertiliser (Gijzen, 2001), and an initial total-P of 15 mg l^{-1}, ABPs will require a 20% larger surface area than required for DBPs. DBPs have a 25% higher area requirement over ABPs, if ponds are to be designed such that the effluent complies with WHO (1989) microbiological quality guidelines for restricted irrigation (<1,000 faecal coliforms/100 ml). A major limitation for the application of algae-based or duckweed-based ponds technology for wastewater treatment at large scale is the large area needed, which is estimated at about 2-4 m^2/capita depending on influent strength of wastewater and effluent guideline requirements. Optimisation of the ABPs and DBPs systems may reduce the area requirement to 1.5-3 m^2/capita making these systems more attractive. However, duckweed stabilisation ponds aim at resource recovery in the light of sustainability of wastewater treatment. Therefore, pond technology should be recommended for rural communities close to agricultural land, where land could be available at reasonable price and the reuse of duckweed and effluent facilitated. This does not exclude urban areas where pond systems for nutrient utilisation can be planned outside the city at convenient locations. An integrated system combining ABPs and DBPs could be a proper solution for nutrients and FC removal at reasonable land requirement (van der Steen *et al*., 1998).

CONCLUSIONS

From the above discussion the following points can be concluded:

- Significantly higher BOD removal efficiency was achieved in DBPs (93-89%) than ABPs (86%). Good correlation between BOD removal rates and BOD surface loading rates in each system was found. This suggests that waste stabilisation ponds are more efficient at higher organic loading. The purification in duckweed system at low and high organic loading conditions led to satisfactory efficiency with respect to reduction of BOD and TSS (20/30 standards) after 28 days HRT, while in ABPs, standards were not achieved.
- Nitrogen removal rates in ABPs were linearly correlated with BOD surface loading rates and nitrogen loading rates, while in DBPs, N-removal rates were almost constant irrespective of BOD surface loading rates and nitrogen loading rates. Overall N-removal rate in algae system was significantly higher than that in duckweed system. Nitrogen uptake by duckweed in DBPs system reduced from 30% of total nitrogen input to the system at low organic loading period to 19% at high organic loading period.
- Similar phosphorus removal rates in the ABPs and in the DBPs were observed at low and high organic loading periods. Phosphorus removal was higher in DBPs than ABPs during the low and high organic loaded periods, due to uptake by duckweed in the former system.
- The effect of light and the higher DO and pH in ABPs than in DBPs resulted in more favourable conditions for the decay of FC in the former system. During both low and high organic loading periods, FC was better removed in ABPs (3.8 and 3.4 log units, respectively) than in DBPs system (2.2 and 1.76 log units, respectively). In ABPs, faecal coliforms were better removed during low organic loading period in comparison with high organic loading period. DBPs behaved likewise.

ACKNOWLEDGEMENTS

The authors are grateful to the Dutch government (SAIL) for financially supporting this research within the collaboration project WASCAPAL between Birzeit University, West Bank and the International Institute for Infrastructural, Hydraulic and Environmental Engineering (IHE), The Netherlands.

REFERENCES

Wong-Chong, G.M. and Loehr, R.C. (1978). The kinetics of Microbial Nitrification: Nitrite-Nitrogen Oxidation. *Wat. Res*.12: 605-609.

Alaerts, G.J., Mahbubar, M.R. and Kelderman, P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res*. 30: 843-852.

Al-Nozaily, F. (2001). *Performance and process analysis of duckweed-covered sewage lagoons for high strength sewage. The case of Sana'a Yemen*. Ph.D. Thesis, IHE, The Netherlands.

Al-Nozaily, F., Alaerts, G. and Veenstra, S. (2000). Performance of duckweed-covered sewage lagoons-II. Nitrogen and phosphorus balance and plant productivity. *Wat. Res*. 34(10): 2734-2741.

Caicedo J. R., van der Steen N.P. Arce O. and Gijzen H.J. (2000). Effect of total nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Wat. Res*. 15: 3829-3835.

Clement, B. and Merlin, G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ*. 170: 71-79.

Copelli, M., Guetti, P.F. and Corradi, M. (1982). Rimozione di azoto e fosforo da acque reflue. Diallevamentisuinicole mediante fitodepurazione. *Rimozione di azoto e fosfore seminario I.R.A.A. 22-23 October 1987*, Rome, 34.

Culley, D. D., Gholson, J. H., Chisholm, T. S., Standifer, L. C. and Epps, E. A. (1978). Water quality renovation of animal waste lagoons utilizing aquatic plants. U.S. Environmental Protection Agency. Ada, Oklahoma, 166 pp.

Curtis, T. P., Mara, D. D. and Silva, S. A. (1992a). Influence of pH, oxygen and humic substances on ability of solar radiation to damage FC in waste stabilisation pond water. *Appl. Envir. Microbilol*. 58(4): 1335-1343.

Curtis, T. P., Mara, D. D. and Silva, S. A. (1992b). The effect of sunlight on faecal coliforms in ponds, implications for research and design. *Wat. Sci. Tech*. 26:1729-1738.

Edwards, P., Hassan, M.S., Chao, C.H. and Pacharaprakiti, C. (1992). Cultivation of duckweeds in septage-loaded earthen ponds. *Bioresource Techn*. 40: 109-117.

Gijzen, H.J. (2001). Anaerobes, aerobes and phototrophs: A winning team for wastewater management. *Wat. Sci. Tech*. 44: 123-132.

Hammer, D.H. and Knight, R.L. (1994). Designing constructed wetlands for nitrogen removal. *Wat. Sci. Tech*. 29: 15-27.

Mandi, L. (1994). Marrakesh wastewater purification experiment using vascular aquatic plants *Eichhornia crassipe* and *Lemna gibba*. *Wat. Sci. Tech*. 29: 283-287.

Mara, D. (1976). Sewage treatment in hot climates. John Wiley&sons. pp. 168.

Metcalf and Eddy (1991). Wastewater engineering. Treatment, disposal and reuse. Tchobanoglous G. and Burton F. L. [eds.], McGraw – Hill. New York.

Nego, V. (1987). Boosting pond performance with aquaculture. *Operation Forum*, 4, 20-24.

Reddy, K. R. and DeBusk, W. F. (1985). Nutrient removal potential of selected aquatic macrophytes. *J. Environ. Qual.* 14: 459-462.

van der Steen, P., Brenner, A., Shabtai, Y. and Oron, G. (2000). The effect of environmental conditions on faecal coliform decay in post-treatment of UASB reactor effluent. *Wat. Sci. Tech*. 42(10-11): 111-118.

van der Steen P., Brenner A., Oron G.; Grabow W.O.K., Dohmann M., Haas C., Hall E.R., Lesouef A., Orhon D., van Der Vlies A., Watanabe Y., Milburn A., Purdon C.D., Nagle P.T. (1998). An integrated duckweed and algae pond system for nitrogen removal and renovation. *Wat. Sci. Tech.* 38(1): 335-343.

Wang, W. (1991). Ammonia toxicity to macrophytes (Common duckweed and rice) using static and renewal methods. *Environm. Toxic. Chem*. 10: 1173-1178.

WHO (1989). *Health guidelines for the use of wastewater in agriculture and aquaculture*. Technical report series 778. World Health Organisation, Geneva, Switzerland.

Zimmo, O. R., Al-Sa'ed, R., van der Steen, P. and Gijzen, H. J. (2000). Nitrogen transformation and treatment efficiency in algae-based and duckweed-based stabilisation ponds. Conference proceeding "*Managing Water and Waste in the New Millennium"*.

Zimmo, O.R., Al-Sa`ed, R. M., van der Steen, N. P. and Gijzen, H. J. (2002). Process performance assessment of algae-based and duckweed-based wastewater treatment systems. *Wat. Sci. Tech*. 45: 91-101.

Zimmo, O. R., Al-Ahmed, M., van der Steen, N. P., Awuah E., Caeciedo E., J. and Gijzen H. J. (Submitted). Comparison of hydraulic flow patterns in algae and duckweed-based continuous flow pilot-scale waste stabilisation ponds.

Zirschky, J. and Reed, S. C. (1988). The use of duckweed for wastewater treatment. *J. WPCF*. 60: 1253-1258.

Chapter 6

COMPARISON OF AMMONIA VOLATILISATION RATES IN ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

Submitted as:

Zimmo O. R., van der Steen N. P. and Gijzen H. J. Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilisation ponds. *Wat. Res*.

COMPARISON OF AMMONIA VOLATILISATION RATES IN ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

ABSTRACT

Quantification of ammonia volatilisation from wastewater stabilisation ponds is important in order to understand the significance of volatilisation for overall nitrogen removal in these widely applied low-cost treatment systems. Ammonia volatilisation rates were measured in pilot plant facilities consisting of one line of four algae-based ponds in series and a parallel line of four ponds with a floating mat of duckweed (*Lemna gibba*) on the water surface. Ammonia volatilisation was assessed during one and a half year period. The method developed is accurate, convenient and is proposed for analysis of a wide range of gasses emitted from stabilisation ponds and possibly other aquatic systems. The ammonia volatilisation rates in algae-based ponds (ABPs) were higher than in duckweed-based ponds (DBPs). This can be explained by the lower values of NH_3 in DBPs due to shading and lower pH values, since the volatilisation rate highly correlated with free ammonia concentration (NH_3) in pond water. The duckweed cover appeared not to provide a physical barrier for volatilisation of unionised ammonia, because whenever NH_3 concentrations were equal in ABP and DBP also the volatilisation rates were equal. Volatilisation was in the range of 7.2 to 37.4 mg-N m^{-2} d^{-1} and 6.4 to 31.5 mg-N m^{-2} d^{-1} in the ABPs and DBPs, respectively. Average influent and effluent ammonium nitrogen measurements showed that the ammonia volatilisation during the study period in any system did not exceed 1.5 % of total influent ammonium. Therefore this study confirmed results from simultaneous experimental work in our laboratory indicating that the nitrification/denitrification process, rather than ammonia volatilisation process, is the most important mechanism for N-removal in ABPs and DBPs.

Keywords: Ammonia volatilisation, algae, duckweed, *Lemna gibba*, stabilisation ponds, wastewater.

INTRODUCTION

Waste stabilisation ponds are low-cost and efficient systems for wastewater treatment, producing high quality effluent that enables water re-use in irrigation (Mara *et al.*, 1992). Recently there is a growing interest in modification of conventional algae-based ponds (ABPs) into so-called duckweed-based ponds (DBPs). These are basically regular ponds with a floating cover of duckweed plants (*Lemnaceae*). For both systems there is a lack of knowledge on the significance of the various nitrogen transformations and fluxes in the system. Quantification of nitrogen transformations is important for proper pond design and production of effluent suitable for disposal or crop irrigation.

In the field of agriculture, several research works on ammonia volatilisation are reported for farmlands, and for dairy and animal slurry concentrated wastewater (Pain *et al.*, 1990; Shilton, 1996; Sommer, 1997; Zhang *et al.*, 1994). Interest in ammonia volatilisation into the atmosphere was triggered because of the effects of ammonia on acid rain, crops and farm workers. Unfortunately very little work has been done on the contribution of wastewater stabilisation ponds to ammonia release into the atmosphere.

Contradictory conclusions were drawn on the importance of ammonia volatilisation from two studies (Pano and Middlebrooks, 1982; Ferrara and Avci, 1982) in which data were obtained from the same stabilisation pond systems. Pano and Middlebrooks

(1982), in accordance with King (1978), Silva *et al*. (1995) and Soares *et al*. (1996), argued that ammonia volatilisation largely explains total nitrogen removal from ponds. However, Ferrara and Avci (1982) claimed that sedimentation of organic nitrogen is the predominant mechanism for N-removal. Unfortunately all these studies are based on theoretical assumptions and not on a complete nitrogen mass balance. Studies for duckweed covered stabilisation ponds based on incomplete mass balance equations assumed that ammonia volatilisation is not very important in overall nitrogen removal (Alaerts *et al*., 1996; Vermaat and Hanif, 1998; Körner and Vermaat, 1998). These authors suggested that main N-removal mechanisms include nitrification/denitrification, duckweed uptake and harvesting. Zimmo *et al*. (2000a) found a nitrogen removal efficiency of 32% and 13% in algae and duckweed batch experiments respectively that was attributed to ammonia volatilisation and denitrification. The importance of ammonia volatilisation in algae and duckweed-based ponds was however not clarified. This paper describes the development and application of a simple method to assess relative contribution of ammonia volatilisation in overall N-removal from waste stabilisation ponds.

MATERIALS AND METHODS

Layout and operation of the pilot plant

This study was carried out in two pilot-scale parallel systems of algae and duckweed-based wastewater stabilisation ponds at the new campus of Birzeit University (BZU), 26 km north of Jerusalem (750 m above s.l). Each treatment system consists of a series of four equal ponds with a total hydraulic retention time of 28 days. The layout of the two treatment pond systems (ABPs and DBPs) is shown in Figure 1. A detailed description of the systems was presented elsewhere (Zimmo *et al*., 2002a). The flow through each system was 0.38 m^3 d^{-1}. Duckweed biomass was harvested every fifth day to restore the density to its initial value of 600 g fresh weight m^{-2}. The maximum duckweed density observed at the best growing conditions was 2300 g fresh weight m^{-2}. The system has been in operation since December 1998. The pilot plant was operated under two different conditions: From Dec. 1998 till the middle of Jul. 2000 wastewater from Birzeit University was used (average influent NH_4^+-N = 60 mg l^{-1}). From the middle of Jul. 2000 till Feb. 2001 wastewater from Al-Bireh city was used (average influent NH_4^+-N = 110 mg l^{-1}).

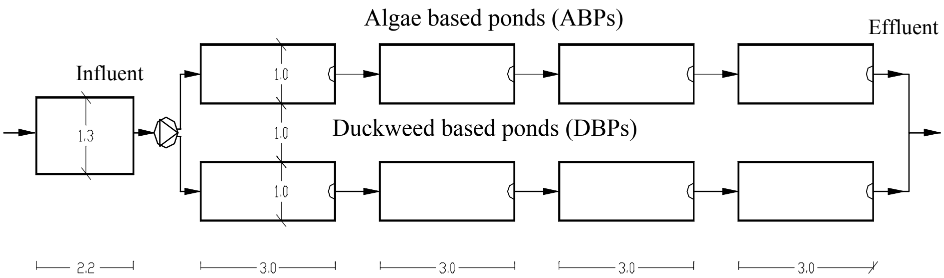


Figure 1. Schematic presentation of the treatment pond systems consisting of 4 algae and 4 duckweed-based stabilisation ponds (HRT=7 d each), preceded by a holding pond (HRT=1 d).

Ammonia volatilisation, pH and water temperature measurements

From October 1999 measurements of ammonia volatilisation were carried out and each measurement covered a 24 h period. Ammonia volatilisation was measured in all four ponds of each treatment system (one pond each day) by placing a Plexiglas transparent box (0.53x0.24x0.35 m) on the pond surface area. One side of the box was open and this side was placed on the pond surface. A constant airflow of 1.5 litre per minute that represents a wind velocity of 1.1 m/hr was maintained through the box using an aquarium air pump. At the gas outlet from the plexiglas box, ammonia gas was trapped in two flasks in sequence, each containing 90 ml 2% boric acid solutions. The tube in the trap flasks was maintained at approximately 1 cm below the acid surface (Figure 2). Boric acid from the traps was collected and analysed for ammonium concentration. Ammonia volatilisation measurements were performed over a period of one and a half year in order to assess seasonal variations. Ammonia volatilisation rates from the total pond surface area were extrapolated from the measured values. A blank control was used to account for ammonia interference from the surrounding air. At the end of each volatilisation measurement, a grab sample from the water column below the box was collected and analysed for ammonium concentration. Pond water temperature and pH underneath the Plexiglas box were measured three times throughout the daytime (morning, afternoon and evening). Pond water pH and temperature were also measured over a 24 hr cycle at 2 hr intervals once every month during the study period.

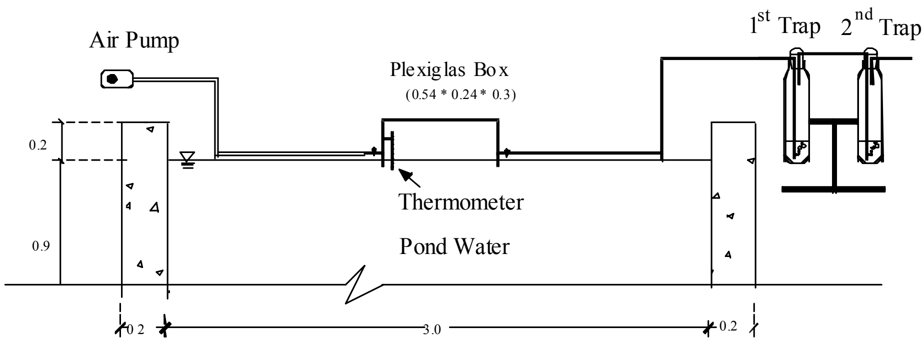


Figure 2: Schematic diagram of experimental set up to establish ammonia volatilisation rates.

Analytical methods

Dissolved oxygen (DO) was measured with a DO175 meter (HACH), pH with an EC10 pH meter (HACH) and temperature with a Mercury thermometer. Ammonium concentration was measured by the Nessler colorimetric method according to the Standard Methods (APHA, 1992).

Theoretical considerations

The proportions of ammonium ion (NH_4^+) and free ammonia (NH_3) are pH and temperature dependent (Erickson, 1985). The unionised ammonia fractions (α) in the pond water were calculated using equation 1 by Clement and Merlin (1995):

$$\alpha = \%\ Unionised\ NH_3 = \frac{100}{1+10^{(pK_a - pH)}} \qquad (1)$$

Unionised NH_3 is relatively volatile and can be removed from solution to the atmosphere via diffusion through water to the surface and through mass transfer from the water surface to the atmosphere. The ammonia volatilisation rate was found to depend on pH, water temperature (Jørgensen, 1989; Stratton, 1968, 1969) and mixing conditions (Pano and Middlebrooks, 1982). Ammonia volatilisation can be described mathematically by the use of the two-film theory of mass transfer in which the water phase is assumed to be well mixed except near the interface. The mass transfer equation (equation 2) with the assumption that the concentration of the ammonia gas in the atmosphere is zero was used to estimate the average ammonia volatilisation rates from each pond of the two systems:

$$N_{NH_3} = -K_l NH_3 \qquad (2)$$

where, N_{NH_3} is the mass transfer rate of ammonia (mg l^{-1} d^{-1}), K_l is the convection mass transfer coefficient in the liquid phase (d^{-1}), NH_3 is the concentration of ammonia in the liquid phase (mg l^{-1}).

This first order equation for ammonia mass transfer rate has been supported by Stratton (1969) who obtained the following expression for the mass transfer coefficient:

$$K_l = \frac{0.0566}{d} \exp[0.13(T - 20)] \qquad (3)$$

where, *d* is the depth of water column in the pond (m) and, *T* is the water temperature (^{0}C)

RESULTS

Ammonia concentrations and environmental conditions

Average influent and effluent NH_4^+-N and corresponding NH_4^+-N removal rates from the ABPs and DBPs during the experimental period are shown in Table 1. Average concentrations decreased throughout the ponds of the two systems and the decrease varied with the season and the wastewater nitrogen content (Table 1). Comparing the pooled volatilisation rates for all duckweed ponds (n=114) with the pooled data for all algae ponds (n=114) showed significant (oneway ANOVA, $P < 0.05$) higher NH_4^+-N removal rates in ABPs during the experimental period. Significantly higher removal rates were obtained when the pilot plant was fed with wastewater of higher ammonium content. In ABPs, most of the time, the pH values were above 8.0 whereas in the duckweed ponds, due to the shading provided by the duckweed cover, lower pH values were observed (Zimmo *et al*., 2002a). During the daytime (only during the warm seasons) the shading provided by the duckweed cover in DBPs caused the temperature in DBPs to be 1 ^{o}C lower than in ABPs (Figure 3).

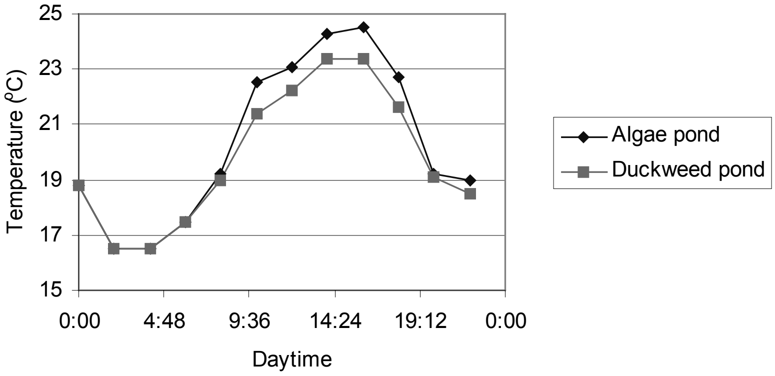


Figure 3. Typical daily variation in water temperature

Table 1. Average influent and effluent NH_4^+-N concentrations (standard deviations) and NH_4^+-N removal rates from the ABPs and DBPs during the experimental period.

	Sample source	Summer 5/6-22/9/99 n=17	Autumn 11/10-18/11/99 n=4	Winter 7/12/99-22/3/00 n=16	Spring 29/3-14/7/00 n=12	Period during 1/8/00-1/2/01* n=17
NH_4^+-N concentration in effl. of DBPs (mg l^{-1})	Influent	60.2 (5.6)	60.4 (4.4)	59.8 (6.4)	60.0 (3.5)	110.0 (9.5)
	D1 effl.	50.5 (5.2)	50.4 (2.2)	52.8 (4.1)	49.3 (2.8)	91.1 (6.4)
	D2 effl.	41.0 (4.1)	41.0 (0.7)	46.1 (4.2)	39.2 (2.1)	73.3 (10.1)
	D3 effl.	32.0 (4.1)	32.1 (1.5)	39.3 (4.1)	29.3 (1.7)	54.4 (9.1)
	D4 effl.	23.7 (4.3)	23.2 (1.6)	32.8 (3.2)	19.8 (1.7)	36.0 (7.1)
NH_4^+-N removal rates (mg-N m^{-2} d^{-1})	Influent-D4 effl.	1174	1197	869	1293	2380
NH_4^+-N concentration in effl. of ABPs (mg l^{-1})	A1 effl.	31.6 (4.0)	34.8 (7.3)	45.2 (5.4)	32.9 (4.5)	85.0 (8.6)
	A2 effl.	20.1 (3.2)	23.4 (2.2)	33.4 (5.4)	23.6 (3.7)	65.7 (9.1)
	A3 effl.	12.5 (1.9)	15.4 (1.9)	23.3 (3.5)	16.9 (3.4)	40.3 (17.5)
	A4 effl.	8.7 (1.9)	9.1 (1.2)	14.6 (2.2)	10.1 (2.8)	23.5 (12.8)
NH_4^+-N removal rates (mg-N m^{-2} d^{-1})	Influent-A4 effl.	1657	1650	1454	1605	2782

n is the number of samples analysed for each pond during the stated period.
* During this period, ABPs and DBPs were fed with raw sewage characterised by high nitrogen content.

Volatilisation rates and effect of environmental factors

Figure 4 shows average ammonia volatilisation rates based on 24 hour measurements in the ABPs and DBPs from June 1999 to July 2000 (a) and from August 2000 to February 2001 (b). During August 2000 to February 2001, when the pilot plant was operated using wastewater from Al-Bireh with higher nitrogen concentration, higher ammonia volatilisation rates were measured in comparison with the previous period of

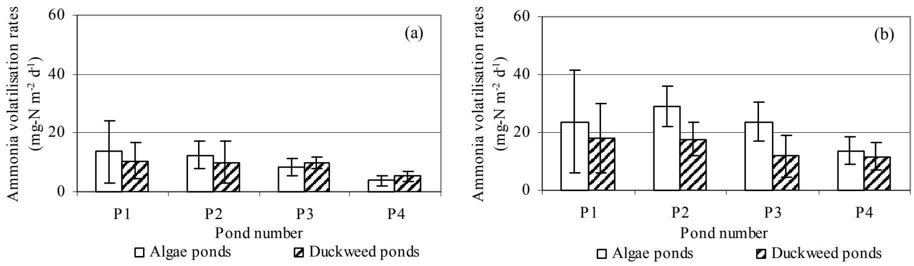


Figure 4. Comparison of average ammonia volatilisation rates in ABPs and DBPs from June 1999 to July 2000 (a) and from August 2000 to February 2001 (b). Number of measurements for each individual pond was 27. The error bars indicate the standard deviations.

operation. Disappearance of duckweed cover from the first three ponds of DBPs was observed when the nitrogen concentrations in the system were increased. NH_3 concentrations of 5 mg-N l^{-1} and higher caused the death of duckweed within 2 days of its exposure to that concentration. Comparable values of ammonia toxicity to duckweed were reported in studies of Wang (1991) and Clement and Marlin (1995) whereas Caicedo *et al*. (2000) reported ammonia toxicity for *Spirodela polyrrhiza* already at lower values. Different species of duckweed may have different sensitivity for ammonia toxicity. Despite the disappearance of duckweed cover, ammonia volatilisation in ABPs was higher than DBPs (Figure 4b). This possibly because in DBPs algae were not yet developed. In individual ponds similar volatilisation rates were obtained at similar ammonium concentrations and similar environmental conditions. Poor correlation (R^2 <0.6) between either pH or water temperature and measured ammonia volatilisation rates in ABPs and DBPs were found. This means that other parameters, rather than pH or water temperature alone, are affecting ammonia volatilisation rates. Better linear correlation factors (R^2 = 0.87-0.90) were obtained when unionised NH_3 concentrations calculated as a function of water temperature, pH and NH_4^+ concentration (Equation 1) were plotted versus the measured ammonia volatilisation rates (Figure 5).

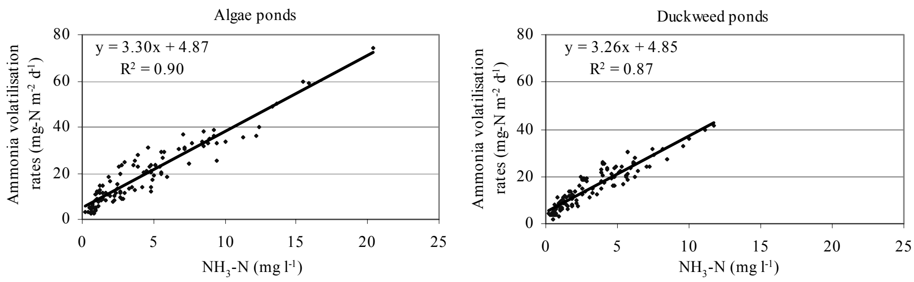


Figure 5. Variation of ammonia volatilisation rate with pond water ammonia concentration in ABPs and DBPs from June 1999 to February 2001.

DISCUSSION

This study describes the development and application of a simple method for direct assessment of ammonia volatilisation rates from stabilisation pond systems treating domestic wastewater. This method can also be used to assess the quantitative contribution of treatment systems to other gas emissions with potential negative environmental impact**.** For verification of the ammonia volatilisation measurement technique, ammonium in the second trap was measured several times during the experimental period. In addition, air from the vicinity of the pond was pumped through the traps. Ammonia concentrations in both cases were negligible. Measuring volatilisation fluxes using this technique appeared to be possible with a simple set-up, which resulted in reproducible results. Therefore it can be assumed that the measured rates are equal to the actual rates of volatilisation occurring under natural quiescent conditions.

The strong diurnal pH fluctuations as occurring in ABPs due to algal photosynthetic activity (CO_2 uptake) was not observed in DBPs (Zimmo *et al*., 2002a). Therefore the average ammonia concentrations in ABPs were higher than in DBPs, since at higher pH the equilibrium between ammonia and ammonium is displaced towards the free form. The higher ammonia concentrations in ABPs were expected to enhance ammonia volatilisation and indeed ammonia volatilisation rates measured in algae-based ponds were significantly higher than those measured simultaneously in duckweed- based ponds. The variation of volatilisation rates over a 24 h period closely followed the variation in pH (data not shown).

Ammonia concentrations not only differed between ABPs and DBPs, but also decreased along the series of ponds. Accordingly the rates of volatilisation decreased along the series of ponds. Similarly ammonia concentrations were lower in winter due to lower pH and temperature, which resulted in lower volatilisation rates during that season. Good correlation coefficients were obtained to describe the relationships between ammonia volatilisation rates and ammonia concentrations (calculated as a function of ammonium concentration, pH and water temperature (equation 1)), whereas poor correlation was observed between volatilisation rates and pH or water temperature alone. This confirms that ammonia concentration is the most important factor affecting the volatilisation rate (Stratton, 1968; 1969).

Whenever ammonia concentrations were the same for any of the algae or duckweed ponds, also ammonia volatilisation rates were similar. Apparently, the duckweed cover did not provide a physical barrier for volatilisation of unionised ammonia. Although the duckweed cover reduces the water surface that is directly exposed to the atmosphere, ammonia volatilisation was not hindered. One possible explanation could be that gas emission from the water phase takes place through the stoma of the plant. This phenomenon has been reported for other gases and plants. For instance, methane emission from rice fields is mainly through the plants (Tyler 1991).

The simulated wind velocity in the Plexiglas box (1.1 m/hr) corresponds to a situation when the air above the pond is nearly stagnant. It is expected that the ammonia volatilisation rates will increase at higher wind velocity as a result of a steeper ammonia gradient caused by the removal of ammonia accumulated in the gas film at the interface. If the measured ammonia volatilisation rates would be corrected for the average highest wind velocity at the pilot plant site according to data obtained by Wachs *et al*. (1972), the volatilisation rates would be two to three times higher than rates indicated in the Figure 4. However, high wind velocities will occur only during the winter season when volatilisation rates were significantly lower.

Our measurements of ammonia volatilisation rates are much lower than values reported for slurry and piggery wastes with higher NH_4^+ and NH_3 concentrations (Pain *et al.*, 1990; Zhang *et al.*, 1994; Shilton, 1996; Sommer, 1997). Our findings are in the same range as reported by Schroeder (1987), Gross *et al.* (1999) and Stratton (1969). They are also in agreement with the conclusion by Ferrara and Avci (1982) who found that ammonia volatilisation in waste stabilisation ponds accounts for only a small fraction of total N-removal from domestic sewage. In duckweed-based ponds, our conclusion on ammonia volatilisation is in agreement with previous reports concluding that volatilisation in duckweed- based ponds is not significant (Alaerts *et al.*, 1996; Vermaat and Hanif, 1998; Körner and Vermaat, 1998; Zimmo *et al.*, 2000a). The volatilisation rates measured in batch incubations of domestic wastewater in buckets with and without duckweed cover (Zimmo *et al.*, submitted) were much higher than measured in the continuously operated pilot plants. This cannot easily be explained but shows that results from short term batch incubations cannot easily be extrapolated to long-term operated pilot plants.

Mechanisms and modelling of ammonia volatilisation

Pano and Middlebrooks (1982) developed an ammonia nitrogen removal model for facultative ponds by developing a relationship similar to the theoretical ammonia stripping model. The authors assumed that nitrification does not account for a significant part of the nitrogen removal because of the low nitrite and nitrate concentrations in the pond water. This assumption is not correct, because low nitrite and nitrate may be due to effective denitrification in the sediment layer (Nielsen, 1992). In fact nitrification/denitrification may represent an important nitrogen flux (Zimmo *et al.*, 2002b). However, Pano and Middlebrooks could model NH_4^+ removal quite accurate with only T and pH as predicting parameters. This is not surprising because pH is correlated with high algae growth that eventually contributes to removal, either via sedimentation or by providing substrate for attachment of nitrifying bacteria in pond water enhancing nitrification and subsequently denitrification (Zimmo *et al.*, 2002b). A model that is more according to our results was proposed by Ferrara and Avci (1982) because it included the different N fluxes. An important shortcoming in their conceptual model is the assumption that nitrification is not taking place. In addition, the model was not validated against data from other pond systems. Their calculation for ammonia volatilisation was based on the assumption that ammonia mass transfer is first order with respect to ammonia nitrogen concentration in the fluid. By using the expression for mass transfer coefficient by Stratton (1969) they concluded that ammonia volatilisation accounts for only a small fraction of total N-removal. Table 2 compares the average rates of ammonia volatilisation from the algae and duckweed-based ponds obtained by direct measurement in the present study with the average rates predicted using the models by Stratton and Pano and Middlebrooks. The predicted rates using these models were calculated for the average pond water pH and temperature measured over a 24 hr duration at 2 hr interval once every month during the study period.

Table 2. Comparison of average ammonia volatilisation (AV) rates predicted using models by Stratton (1969) and Pano and Middlebrooks (1982) with rates obtained by direct measurement (this study). Average values over the study period of influent and pond water ammonia concentrations, T and pH (daily average) are given.

Pond identification		NH_4^+-N mg l^{-1}	NH_4^+-N mg l^{-1}	T	pH	Fraction NH_3-N	NH_3-N	Stratton	Pano and Middlebrooks	Measured in this study
		In/out	Pond water	^oC	(-)	%	mg l^{-1}	AV rates in mg m^{-2} d^{-1}		
ABPs	Inf.	70.1								
ABPs	A1	46.0	59.1	16.7	8.1	3.8	2.2	81.1	3607	21.7
ABPs	A2	32.4	43.7	17.0	8.1	3.6	1.6	60.5	2399	22.3
ABPs	A3	19.4	27.3	17.3	8.0	3.0	0.8	37.7	1666	19.1
ABPs	A4	11.4	18.3	17.1	8.0	2.8	0.5	19.8	1021	10.5
DBPs	D1	58.4	67.4	16.5	8	3.0	2.0	63.0	3199	18.1
DBPs	D2	47.7	52.6	17.3	7.8	2.0	1.1	41.8	2203	16.4
DBPs	D3	37.0	43.4	16.9	7.8	1.7	0.8	28.3	1765	14.2
DBPs	D4	26.8	31.7	16.5	7.7	1.5	0.5	17.7	1180	10.6

Predicted values by the Stratton model were one to two times higher than the measured values, but values become similar when corrected for wind effects. Ammonium removal rates predicted by the Pano and Middlebrooks model were much higher than the measured values in our experiments. A possible explanation for that was discussed above. Our results suggest that stabilisation ponds are probably more environmentally friendly treatment systems than one might expect from results of Pano and Middlebrooks who suggested extreme high values for ammonia release and subsequent negative impact on the environment. Based on our measurements for ammonia volatilisation from ABPs and DBPs, the following equation is proposed to calculate the amount of ammonia volatilisation from stabilisation ponds:

$$Y = 3.30\ x + 4.90$$

where,
Y is the ammonia volatilisation in mg-N m^{-2} d^{-1},
x is the calculated NH_3 (mg-N l^{-1}) as a function of pH, water temperature and ammonium concentration in pond water (equation 1).

The nitrogen removal via ammonia volatilisation in ABPs and DBPs were corresponding to less than 1.5 % of total influent ammonium to the treatment systems. If wind effects are taken into account the relative contribution of ammonia volatilisation may account for 2-3%. Therefore, ammonia volatilisation was not the major mechanism for ammonia removal in the pilot plant used in this study. This suggests that ammonia volatilisation is insignificant for nitrogen removal in natural treatment systems with wastewater characteristics and environmental conditions similar to the present experimental set up. However, if the average pond water pH would rise to 9, the ammonia volatilisation is expected to account for 21% of the nitrogen mass balance.

This study clarifies the contradicting conclusions that others reached about the role of ammonia volatilisation in overall nitrogen removal from algae and duckweed-based ponds. The investigation of other nitrogen fluxes in the two systems through sedimentation, nitrification and denitrification and plant uptake is subject of current research in our laboratory. The method used in this work can be adjusted to measure CH_4, H_2S, NO_x, CO_2 and other gas emissions from anaerobic, facultative and maturation ponds with a view to evaluate the secondary environmental impacts of such treatment ponds.

CONCLUSIONS

The present study demonstrates that the ammonia volatilisation was highly correlated with NH_3 concentration in pond water, which in turn was governed by the combined effect of pH and water temperature. Shading provided by the duckweed cover prevented strong diurnal changes in pond water pH such as commonly observed in conventional stabilisation ponds. The lower pH in DBPs in comparison with ABPs resulted in lower ammonia concentrations and hence in lower ammonia volatilisation. However similar ammonia volatilisation from ABPs and DBPs at equal ammonia concentrations was observed. Apparently the physical barrier provided by the duckweed cover did not hinder ammonia volatilisation. Volatilisation rates decreased along the series of ponds as the unionised ammonia concentration decreased. Ranges of 7.2 to 37.4 mg-N m^{-2} d^{-1} and 6.4 to 31.5 mg-N m^{-2} d^{-1} were observed in ABPs and DBPs, respectively. Nitrogen removal via ammonia volatilisation in the algae and duckweed ponds at calm conditions did not exceed 1.5 % of total influent ammonium. This suggests that other nitrogen removal mechanisms such as nitrification/denitrification or sedimentation may be more important in overall nitrogen removal. The method for the measurement of ammonia volatilisation as developed and applied in this research could be adjusted for the quantification of wide range of different gas fluxes (such as CH_4, H_2S, NO_x and CO_2) from stabilisation pond systems. The method therefore maybe useful to assess and compare environmental impacts from wastewater treatment systems due to (green house and acid rain) gas emissions.

ACKNOWLEDGEMENTS

This work was financed by the Netherlands within the scope of the collaboration project "Water Sector Capacity Building in Palestine" between Birzeit University-West Bank and UNESCO-IHE Institute for Water Education, The Netherlands. The authors like to thank the laboratory assistant Marwan Ayad for his support.

REFERENCES

Alaerts G.J., Mahbubar M.R., Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res.* **30:** 843-852.

APHA (1992) Standard methods for examination of water and wastewater. 18th edition, *Am. Publ. Assoc*., Washington D.C., USA.

Caicedo J. R., van der Steen N.P. Arce O. and Gijzen H.J. (2000). Effect of total nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Wat. Res.* **15:** 3829-3835.

Clement B. and Merlin G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ*. **170:** 71-79.

Erickson J.R. (1985). An evaluation of mathematical models for the effect of pH and temperature on ammonia toxicity to aquatic organisms. *Wat. Res*. **19:** 1047-1058.

Ferrara R. A.and Avci C. B. (1982). Nitrogen dynamics in waste stabilisation ponds. *J. WPCF*. **54:** 361-369.

Gross A., Boyd C.E. and Wood C.W. (1999). Ammonia volatilisation from freshwater fish ponds. *J. Environ. Qual*. **28:** 793-797.

Jørgensen S. E. (1989). Sedimentation. pp 109-124. In: S.E. Jørgensen and M.C. Gromiec (Eds.). Mathematical submodels in water quality systems. Developments in environmental modelling. Elsevier Publishers B.V.

King D.L. (1978). The role of ponds in land treatment of wastewater. *Proc. International Symposium on Land Treatment of Wastewater*, Hanover, N.H. 191.

Körner S. and Vermaat J. E. (1998). The relative importance of *Lemna gibba* L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Wat. Res*. **32**: 3651-3661.

Mara D.D., Alabaster G.P., Pearson H.W. and Mills S.W. (1992). *Waste stabilisation ponds: a design manual for eastern Africa*. Lagoon Technology International, Leeds.

Nielsen L.P. (1992). Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiol. Ecol*. **86:** 357-362.

Pain B.F., Misselbrook T.H., Clarkson C.R. and Rees Y.J. (1990). Odour and ammonia emissions following the spreading of anaerobically-digested pig slurry on grassland. *Biological Wastes* **34**: 259-267.

Pano A. and Middlebrooks E.J. (1982). Ammonia nitrogen removal in facultative wastewater stabilisation ponds. *Journal of Water Pollution Control Federation,* **54**: 344-351.

Schroeder G.L. 1987 Carbon and nitrogen budgets in manured fish ponds on Israel's coastal plain. *Aquaculture* **62**: 259-279.

Shilton A. (1996). Ammonia volatilisation from a piggery pond. *Wat. Sci. Tech*. **33:** 183-189.

Silva S.A., de Pliveira R., Soares J., Mara D.D. and Pearson H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Wat. Sci. Tech*. **31:** 321-330.

Soares J., Silva S.A., de Oliveira R., Araujo A.L.C., Mara D.D. and Pearson H.W. (1996). Ammonia removal in a pilot-scale WSP complex in Northeast Brazil. *Wat. Sci. Tech*. **33:** 165-171.

Sommer S.G. (1997). Ammonia volatilisation from farm tanks containing anaerobically digested animal slurry. *Atmospheric Environment*. **31:** 863-868.

Stratton F.E. (1968). Ammonia nitrogen losses from streams. *Jour. San. Div., Amer. Soc. Civil Engr*., **94:** SA6, 1085-1092.

Stratton F.E. (1969). Nitrogen losses from alkaline water impoundments. *Jour. San. Eng. Div., Amer. Soc. Civil Engr*., **95:** SA2.,223-231.

Tyler S. T. (1991). The global methane budget. In: Rogers J.E. and Whitman W. B. 1991 (eds.). Microbial production and consumption of greenhouse gases: Methane, nitrogen oxides and halomethanes. *American Society for Microbiology* Washington D.C. pp 7-38.

Vermaat J. E.and Hanif M.K. (1998). Performance of common duckweed species (*Lemnaceae*) and the *Waterfern Azolla Filiculoides* on different types of wastewater. *Wat. Res*. **32:** 2569-2576.

Wachs A.M., Folkman Y. and Shemesh D. (1972). Use of surface stirrers for ammonia desorption from ponds. Sixth conference of the IWPRA on Application of New Concepts of Physical-Chemical Wastewater Treatment, Sept. 18-22, Jerusalem.

Wang W. (1991). Ammonia toxicity to macrophytes (Common duckweed and rice) using static and renewal methods. *Environm. Toxic. Chem*. **10:** 1173-1178.

Zhang R.H., Day D.L., Christianson L.L. and Jepson W.P. (1994). A computer model for predicting ammonia release rates from swine manure pits. *J. Agric. Engng Res*. **58:** 223-229.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2000a). Comparison between algae-based and duckweed-based wastewater treatment: differences in environmental conditions and nitrogen transformations. *Wat. Sci. Tech*. **42:** 215-222.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2002a). Process performance assessment of algae-based and duckweed-based wastewater treatment systems. *Wat. Sci. Tech*. **45:** 91-101.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2002b). Quantification of nitrification and denitrification rates in algal and duckweed based wastewater treatment systems. *Conference proceeding 5th International IWA specialist group conference on Waste Stabilisation Ponds.* "*Pond technology for the new millennium*" 2-5 April 2002, Auckland, New Zealand.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2000b). Nitrogen transformation and wastewater treatment efficiency in algae-based and duckweed-based stabilisation ponds. *Conference proceeding "Managing Water and Waste in the New Millennium"*.

Zimmo O.R., van der Steen N.P. and Gijzen H.J. (submitted). Effect of organic surface load on process performance of pilot scale algae and duckweed-based waste stabilisation ponds.

Chapter 7

QUANTIFICATION OF NITRIFICATION AND DENITRIFICATION RATES IN PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

Submitted as:

Zimmo O. R., can der Steen N. P. and Gijzen H. J. Quantification of nitrification and denitrification rates in pilot-scale algae and duckweed-based waste stabilisation ponds. *Environ. Technol*.

Chapter 7

QUANTIFICATION OF NITRIFICATION AND DENITRIFICATION RATES IN PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTE STABILISATION PONDS

ABSTRACT

Nitrification and denitrification rates at three different depths (0.1, 0.45 and 0.9m from the water surface) in two series of four algae and duckweed-based waste stabilisation ponds (ABPs and DBPs) were measured using nitrate reduction techniques in laboratory batch incubations. Effect of temperature and BOD loading was investigated. In situ measurements over the ponds' depth were also done for confirmation of laboratory results. Higher DO concentrations in ABPs, especially during warm season, favoured higher nitrification in ABPs over DBPs. Organic surface loading also affected the rate of nitrification in the pond. Nitrification rates did not increase along the treatment line despite the decreasing in organic matter content. Adsorption of nitrifiers to available suspended particles and subsequent sedimentation is expected to be the main reason for the similar nitrification rates in most ponds. In both systems, the presence of DO in the water column resulted in very low denitrification rates (5-45 mg-N m^{-2} d^{-1}). Higher denitrification rates (160-560 mg-N m^{-2} d^{-1}) were measured in the sediments where anaerobic conditions prevailed. The absence of nitrite or nitrate accumulation suggests sufficient nitrite and nitrate diffusion within the water column to allow full denitrification. The nitrification and denitrification rates in both systems were higher at high temperature. The range of nitrogen removal efficiency via denitrification in ABPs and DBPs corresponded to 15-25% of total influent nitrogen.

INTRODUCTION

Wastewater treatment plants are generally designed to remove organic load, nutrients and pathogens from the waste stream. As legislative pressure from local and international organisations on effluent quality increases, low levels of nutrients are required. Nitrogen is a major nutrient in wastewater that must be reduced to acceptable levels. Its removal in wastewater treatment is very important because of eutrophication in receiving bodies and groundwater contamination. Nitrogen removal could be achieved by nitrification and denitrification within activated sludge systems, by break point chlorination or by ion exchange, but the investment and operational costs are high. Recently, removal of nitrogen in low cost conventional algae-based ponds (ABPs) and duckweed-based ponds (DBPs) is given some attention. In ABP systems, most of nitrogen removal was attributed to ammonia volatilisation (King, 1978; Pano and Middlebrooks, 1982; Silva *et al*., 1995; Soares *et al*., 1996), however, field measurements revealed that this mechanism is not important (Zimmo *et al*., submitted). The role of nitrification and denitrification in these systems is not clear and contradictory results are reported. Ferrara and Avci (1982) stated that ample evidence has been presented in the literature demonstrating that nitrification does not occur in stabilisation ponds under normal conditions. Most authors presume previous assumption because low concentrations of nitrite and nitrate were found in the effluent. However, low concentrations of both nitrite and nitrate do not prove that nitrification does not occur in ponds (Reed, 1985) and in fact may be due to efficient denitrification. In some studies in macrophyte systems, however, ammonia volatilisation was assumed to be negligible and denitrification was referred to as unaccounted for nitrogen and found to contribute for 10-20% to the nitrogen removal (Alaerts *et al*., 1996; Körner and Vermaat, 1998; Vermaat and Hanif, 1998). In order to assess the actual contribution of nitrification and denitrification to overall N-removal in pond systems two approaches can be taken: a- measurement of all the N-fluxes and transformations and assuming the unaccounted to be denitrification. b- Direct measurement of nitrification and denitrification rates. The latter approach was taken in this study. This study is part of a research project on nitrogen transformations in pilot-scale ABPs and DBPs. The aim of this paper is to measure and compare the nitrification and denitrification rates in both systems.

MATERIAL AND METHODS

Description of Pilot Plant and Experiments

Three types of experiments to measure nitrification and denitrification rates in pilot-scale ABPs and DBPs at Birzeit University (BZU)-Palestine were conducted. The pilot plant consisted of a holding tank followed by two parallel treatment systems of duckweed and algae-based ponds operating in series of four ponds each. Each pond has a depth of 0.9 m and a surface area of 3 m^2. The residence time was 7 days in each pond and a total of 28 days for each system. Further details on the pilot system are given elsewhere (Zimmo *et al*., 2000). The pilot plant (Figure 1) was fed with wastewater from BZU and monitored during three periods.

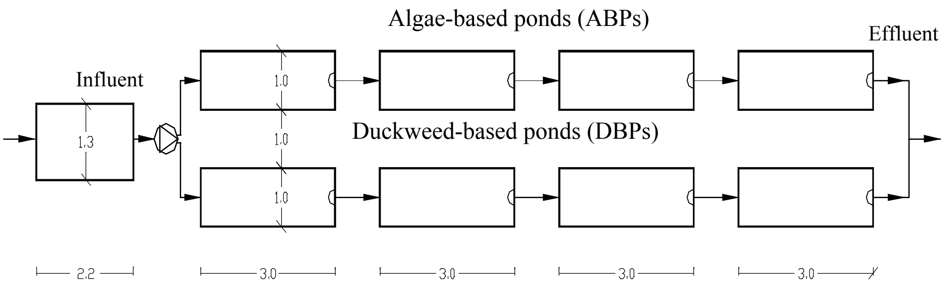


Figure 1. Schematic presentation of the treatment pond systems consisting of 4 algae and 4 duckweed-based waste stabilisation ponds (HRT=7 d each) preceded by a holding pond (HRT=1 d).

Period 1 and 2 were from December 1999-August 2000 (period 1 during cold season from December 1999-March 2000 and period 2 during warm season from June-August 2000) when the plant was fed with wastewater of low organic strength according to Metcalf and Eddy (1991). Period 3 was from May–July 2001 when the plant was fed with wastewater of high organic strength. Results obtained for treatment parameters during the three periods of operation are presented in Table 1.

Table 1. Total-N, NH_4^+-N and BOD in influent and effluent of the four algae (A) and duckweed (D) ponds (P) during the three periods of monitoring (n=14). Typical coefficients of variation were 5-20% around the values shown. All values in mg l^{-1}. In = influent.

		Period 1					Period 2					Period 3				
		In	P1	P2	P3	P4	In	P1	P2	P3	P4	In	P1	P2	P3	P4
Total-N	A	62	52	42	32	26	61	47	35	26	17	69	55	43	33	25
	D		54	47	40	33		53	42	32	23		59	47	38	29
NH_4^+-N	A	60	45	33	23	14	59	34	23	17	9	62	42	31	24	17
	D		52	45	38	32		49	39	29	19		51	41	33	25
BOD	A	149	80	44	34	27	167	82	48	36	23	363	203	127	76	49
	D		74	41	27	18		61	25	17	12		197	104	68	41

In this study actual rates of nitrification and denitrification were quantified in a number of water and sediment samples. In the water column, samples were taken at three different depths. Segment 1 and 2 presented the water phase (0-10 and 40-45 cm depth, respectively) and segment 3 presented the sediment phase (80-90 cm depth). Quantification of rates in the water phase (experiment 1) and the sediment phase (experiment 2) were carried out on samples incubated in the laboratory. Besides, in situ measurements of the rates were performed in pond column incubations (experiment 3).

Experiment 1

Water samples were collected from segment 1 and 2 of the water phase from each pond of the two systems by gently immersing a tube of the peristaltic pump at the corresponding depth and approximately one litre of wastewater was collected. Seasonal effects as well as the effect of organic load on nitrification and denitrification were tested during the three periods of pilot plant operation. For water sample incubations, plastic containers of 4.5 cm diameter and 3.5 cm depth (100 ml volume) were used. For the incubations from segment 1 of duckweed ponds, duckweed from an equivalent surface area of the incubation containers (4.5 cm diameter) was shaken in the water sample and roots were cut and added to it.

Experiment 2

Sediment samples from the ABPs and DBPs were collected after evacuating the sediment and overlaying water column inside a 2.3 cm glass tube immersed into the sediment layer. After water decantation, the sediment sample was poured in a 100 ml-graduated cylinder that has approximately the same cross sectional area of the tube that was used to withdraw the sediment. Decanted overlaying water was poured carefully over the sediment samples up to a total volume of incubation of 100 ml (water depth approximately 17 cm). For analysis of nitrogen compounds in the incubations, samples were taken from the water phase.

Experiment 3

In situ measurements of nitrification and denitrification rates for the whole water column including the sediment were conducted. PVC pipes of 0.1 m diameter and 1 m length were used. Pipes were slowly immersed in the pond and pushed into the sand layer at the pond bottom isolating a volume of 7.1 litre of water from the surrounding. Incubation of the isolated volume was carried out for 24 hour.

Incubation conditions: Since the aim of this study is to quantify actual nitrifying rates in the water and sediment layer in pond systems, attempt was made to mimic in situ temperature and light in our laboratory incubations. The incubation of samples for experiments 1 and 2 was conducted in the laboratory in an incubator pre-set at the in situ pond temperature measured at the time of sample collection. Water samples were illuminated with fluorescent lamps with a photoperiod of 12 hours. KNO_3 was added to increase the nitrate concentration in the sample to approximately 5-7 mg NO_3^-- N l^{-1} in all incubations. This was done because NO_3^- concentration in the samples drawn from the pond was that low (1-2 mg l^{-1}) and that NO_3^- could be consumed before the end of the incubation period. The addition of NO_3^- will not affect nitrification rates because in general the two reactions for nitrification (ammonia oxidation and nitrite oxidation) are zero order reactions (Wong and Loehr, 1978). DO concentration in the sediment was found to be zero, before pre-incubation, sodium sulphite (Na_2SO_3) was added to the samples to simulate in situ anoxic conditions. pH was adjusted to values measured in situ. Dissolved oxygen, pH and temperature were measured at the beginning and the end of the incubation. Each sample was incubated under two different conditions (with and without nitrification inhibitor). Nitrification inhibitor (2 mg of 1-allyl-2-thiourea (ATU), MERCK) was added to measure denitrification rates without the influence of nitrifiers. Each sample was incubated in triplicate for 24 hours. Evaporation losses were replenished by addition of distilled water and water samples were analysed for NO_2^- and NO_3^-.

Determination of denitrification rates: The approach for determining denitrification is based on the consumption of nitrate (Andersen, 1977). Denitrification rates were determined by considering the disappearance of NO_x^- concentrations over 24 hour period incubation with nitrification inhibitor. The nitrification inhibitor blocked ammonium transformation to nitrate and the reduction of nitrate was attributed to the denitrification process.

$$Denitrification\ rate = \frac{\left[[NO_x^- - N]_{initial} - [NO_x^- - N]_{final}\right]_{with\ inhibitor}}{Incubation\ period,\ d}$$

where $[NO_x^- \text{-N}]_{initial}$ and $[NOx^- \text{-N}]_{final}$ are the initial and final (after one day) nitrite and nitrate concentrations in sample incubations with nitrification inhibitor, respectively.

Determination of nitrification rates: The approach for determining nitrification rates is based on determining the NO_x^- as a measure of NH_4^+ oxidation rate (Belser and Mays, 1982). As nitrification and denitrification in a heterogeneous environment may occur simultaneously, the nitrate concentration observed is the net result of the two processes. To measure the actual rate of nitrification, it is essential to take into account the rate of denitrification that would take place (see above). Nitrification rates were determined as the increase in NO_x^- concentrations over 24 hour period. The following equation was used:

$$Nitrification\ rate = \frac{\left[[NO_x - N]_{final} - [NO_x - N]_{initial}\right]_{without\ inhibitor}}{Incubation\ period,\ d} + [Denitrification\ rate]_{with\ inhibitor}$$

where $[NO_x^- \text{-N}]_{final}$ and $[NO_x^- \text{-N}]_{initial}$ are the initial and final (after one day) nitrite and nitrate concentrations in sample incubations without nitrification inhibitor, respectively.

Analytical Methods

Nitrite and nitrate were analysed according to advanced water quality laboratory procedures manual by HACH. Dissolved oxygen, pH and temperature were measured using specific electrodes. DO was measured using DO175 (HACH). Temperature and pH values were measured using EC10 pH meter (HACH). BOD was analysed according to standard methods (APHA, 1992).

RESULTS

Dissolved oxygen in the water column was higher in ABPs than DBPs. Generally DO was highest at the top layer in all ponds. It decreased at increasing distances from the surface and anaerobic conditions were found in the sediments for all ponds (Figure 2). In ABPs mean temperatures of 10, 20 and 21 ^{o}C were measured during period 1, 2 and 3, respectively. Average BOD for the influent and effluent from each pond during period 1,2 and 3 was shown in Table 1. A reduction of 81%, 86% and 86% was found in ABPs for the three periods, respectively. While in DBPs higher reductions were observed (87%, 93% and 89%, respectively).

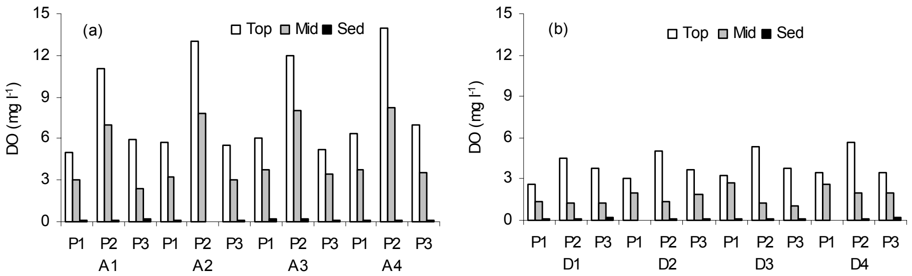


Figure 2. DO concentrations at the top, in the middle and just above the sediment during the three periods of monitoring (P1, P2 and P3) of ABPs (a) and DBPs (b). Coefficients of variation were 3-60% around the values shown above.

Laboratory Determination of Nitrification and Denitrification rates

Incubated water samples were always aerobic (DO> 1 mg l^{-1}) till the end of the incubation, whereas sediment samples were anaerobic throughout. The pH in the incubations was adjusted to in situ values and remained constant during the incubation. In the presence of the nitrification inhibitor nitrate concentration was stable or decreased during the incubation, for samples from the water column and the sediment, respectively. Nitrate concentrations, however, did not reach values below 4 mg-N l^{-1}. Similarly, without nitrification inhibition the nitrate concentration was constant or increased in the water samples and decreased in the sediment samples.

It was found that environmental conditions were similar within the following three depths of each pond: 0 to 37, 37 to 73 and 73 to 90 cm (data not shown). Therefore, it was assumed that the measured rates by incubation were representative average values for the top layer (37 cm), the middle layer (37 cm) and the bottom layer (17 cm, including sludge), respectively. The volumetric rates (in mg l^{-1} d^{-1}) were converted to the surface rates (in mg m^{-2} d^{-1}) by multiplying the measured rates with the layer thickness. Results for the various ponds and experimental periods are given in Figure 3 and 4. In general nitrification rates are not significantly different (oneway ANOVA, p>0.05) when comparing any ABP with the corresponding DBP during the same experimental period. The nitrification rates in period 1 differed significantly (oneway ANOVA, p<0.05) from the rates in period 2 for each individual pond of the two systems. Nitrification rates in period 3 differed significantly from the rates in period 2 only in the first algae pond and first and second duckweed ponds. Denitrification rates in ABPs differed significantly from the rates in DBPs during period 2. The denitrification rates during period 1 in all the ABPs differed significantly from the rates during period 2. In the DBPs no significant difference between these periods was observed. During period 2 and 3 denitrification rates were significantly different for the second and fourth algae pond, but similar rates were observed in duckweed ponds. The nitrification rates from the 4 ponds in each system lumped together were significantly different during period 2 and 3 and the denitrification rates during period 2.

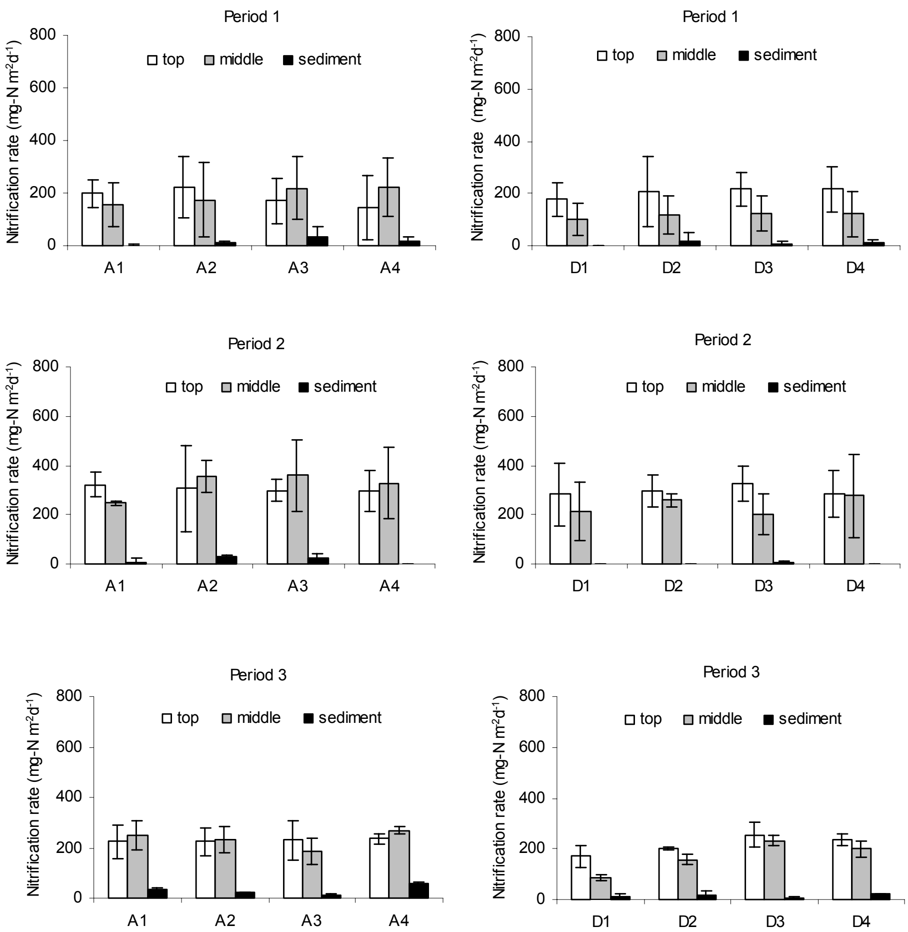


Figure 3. Nitrification rates (in mg-N m^{-2} d^{-1}) in the 10, 45 and 90 cm depth water and sediment samples at each pond of the two systems (ABPs and DBPs) for the three experimental periods. Error bars represent standard deviation for 7,5 and 4 samples during period 1, 2 and 3, respectively.

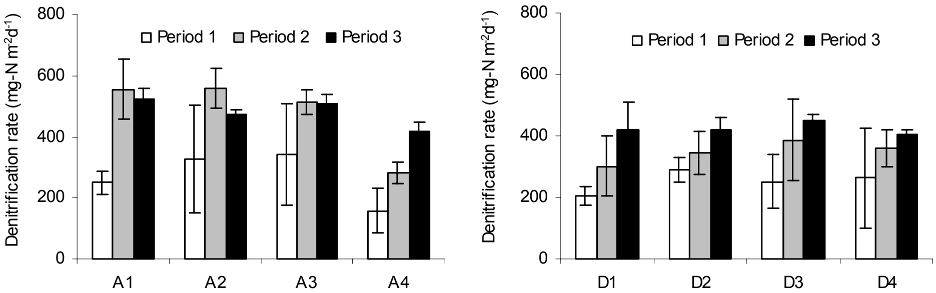


Figure 4. Denitrification rates (in mg m^{-2} d^{-1}) in the sediment samples at each pond of the two systems (ABPs and DBPs) for the three experimental periods. Error bars represent standard deviation for 7,5 and 4 samples during period 1, 2 and 3, respectively.

In situ Measurement of Nitrification and Denitrification Rates

The nitrification and denitrification rates measured in the in situ experiments in pond vertical columns that were enclosed in the PVC pipe are presented in Table 2. Net nitrification rates present the difference between nitrification and denitrification rates. The nitrate concentrations during the experiments followed the same pattern as observed in the laboratory incubations. The NO_x concentration in the water samples decreased with 20 to 70% of the initial concentration in the presence of the nitrification inhibitor, but nitrate was not exhausted before the end of the experiment. NO_x concentrations in the water samples increased in the incubations during period 1 and 2 (range: 1 to 7%), but slightly decreased in some of the incubations during period 3, even in the incubations without inhibitor. Average nitrification rates in ABPs during the three experimental periods were respectively 16, 42 and 38% higher than rates in DBPs. Denitrification rates were also higher in ABPs by 24, 43 and 39%.

Table 2. In situ nitrification and denitrification rates and corresponding net nitrification rates in ABPs and DBPs during the three experimental periods (n for each pond is 12, 4 each experimental period). Typical coefficients of variation were 4-20% around the values shown.

Pond ID	Nitrification rates mg-N m^{-2} d^{-1}			Denitrification rates mg-N m^{-2} d^{-1}			Net nitrification mg-N m^{-2} d^{-1}		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
A1	431	530	309	397	513	321	34	17	-13
A2	480	587	447	461	539	413	19	48	35
A3	548	623	552	491	589	504	57	35	48
A4	204	377	485	180	334	437	24	43	48
D1	330	286	264	265	265	272	64	20	-8
D2	426	414	271	358	374	284	68	40	-13
D3	336	434	379	315	409	318	22	25	61
D4	334	352	386	293	335	332	41	17	53

DISCUSSION

The measurement of nitrification and denitrification rates by incubating pond samples under controlled conditions in the laboratory has been shown to be a simple but reliable method that produced reproducible results. The measured rates in the laboratory are aimed to predict the actual in situ rates. The potential nitrification and denitrification rates under ideal environmental condition were not measured in this study. The rates measured in the incubations were confirmed by slightly higher rates observed in situ. However, disturbance of samples during collection can affect concentration gradients that may have existed in situ and the disturbance may have activated inactive cells. Comparison of the applied method with ethylene inhibition (Revsbech *et al.*, 1988) or ^{15}N tracer techniques (Goeyens *et al.*, 1987) or direct measurement of the N_2 flux (Seitzinger *et al.*, 1980) is suggested.

Effect of Experimental Variables

The incubations showed that nitrification rates in ABPs are similar in the top and middle layer, but negligible in the sediment and the 17 cm water layer just above the sediment. The absence of nitrification in the bottom layer is probably due to the lack of oxygen. Nitrification took place in all locations where DO values were higher than the critical threshold of 0.5 mg l^{-1} reported by Taylor and Bishop (Taylor and Bishop, 1989). During period 2, the equal rates in the top and middle layer of algae ponds are not surprising since DO concentration (8 mg l^{-1} in the middle layer and 12 mg l^{-1} in the top layer) was above the half saturation coefficient. Apparently DO was not limiting for nitrification in both the top and middle layers. DO-nitrification rate relationship in the ABPs and DBPs during the three periods of monitoring (Figure 5) fits reasonably well with a Monod model with half saturation coefficient of 1.7 mg O_2 l^{-1}. This is close to reported half saturation constants by Wiesmann (Wiesmann, 1994) for ammonium oxidizers and nitrite oxidizers of 0.3 and 1.1 mg O_2 l^{-1}, respectively.

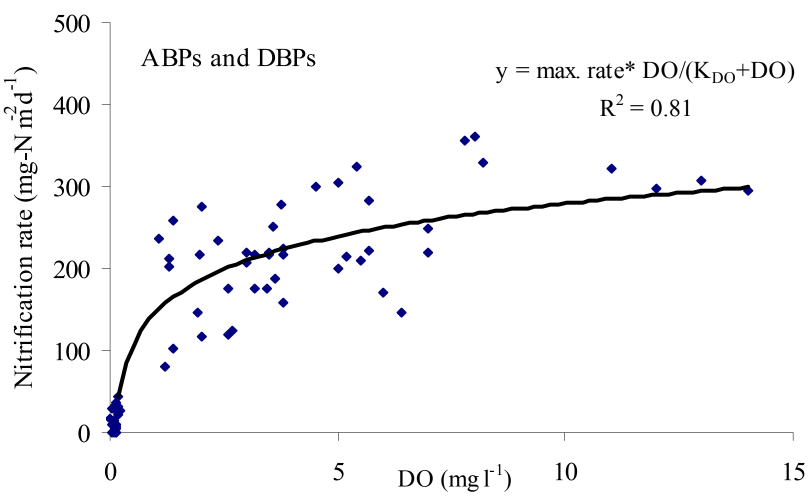


Figure 5. Plot of nitrification rates versus corresponding DO concentration in ABPs and DBPs during the three periods of monitoring.

The nitrifying activity in the water column and in the sediment in our systems appeared to be autotrophic in nature. During sample incubations with the nitrification inhibitor, the ammonium oxidation was inhibited by the presence of ATU (only 2% reduction in ammonium). This inhibitor was found to be a strong selective inhibitor of ammonia oxidation (Ginestet *et al*., 1998). In reality heterotrophic nitrification in the sediment could take place by heterotrophic nitrifying bacteria that can oxidise both inorganic and organic nitrogen compounds (Van Luijn 1997; Verstraete and Alexander, 1973) at low oxygen concentration (Laurent, 1971 cited by Green *et al.*, 1996). Therefore, despite better growth of heterotrophs in the sediment, autotrophic nitrification appears to be more important in overall nitrification. This is also in agreement with Henriksen and Kemp (1988).
Comparing rates in ABPs for the first and second experimental periods (10 and 20 ^{0}C respectively) shows an average increase by 64%. The same temperature increase in activated sludge systems results in an average increase of nitrification rate by 166% (Metcalf and Eddy, 1991). The nitrification rates in algae ponds were also affected by the organic surface load. A 100% increase in the influent BOD concentration during period 3 resulted in higher loading rate in the first pond of the two systems. DO concentration dropped below the half saturation coefficient in the first pond of the two systems and resulted in lower nitrification rates in comparison with period 2. In the rest of the ponds, DO was still higher than the half saturation coefficient and nitrification rates showed similar values as in period 2. Nitrification rates of up to 200 mg-N m^{-2} d^{-1} were obtained at organic loading of 467 kg-BOD $ha^{-1}d^{-1}$ and higher rates (300-400 mg-N m^{-2} d^{-1}) were obtained at organic loading between 30-100 kg-BOD $ha^{-1}d^{-1}$.

Mechanisms and Limiting Factors

Nitrifiers are known to prefer attachment to solid surfaces (Focht and Verstraete, 1977; Underhill and Prosser, 1987; Verhagen and Laanbroek, 1991). In the algae pond environment this is limited to suspended solids, algae biomass and sidewalls. It seems therefore, also based on the previous observations, that limiting for the increase in number of nitrifying bacteria in the top and middle layer of algae ponds is attachment. Nitrification did not increase along the treatment line although this was expected due to decrease in organic matter content. It is not likely that the number of nitrifiers in the water phase would increase in any of the ponds because nitrifiers that attach to disperse biomass or are present in suspension eventually settled to the anaerobic bottom where it would either die or become inactivated due to unfavourable conditions. Therefore, lack of surface for adsorption of nitrifiers in the pond water column is likely limiting for nitrifying microorganisms to remain in the water phase and high nitrification rates to occur. Inhibition of nitrifiers by free ammonia has been well documented in literature. Depending on ammonium concentration, pH and water temperature, free ammonia of 0 to 9 mg NH_3 l^{-1} was calculated in ABPs and to a lesser extent in DBPs (0 to 6 mg NH_3 l^{-1}) (Zimmo *et al*., Submitted). Abeling and Seyfried (1992) reported that concentrations of 1-5 mg NH_3-N l^{-1} inhibited oxidation of nitrite to nitrate by *Nitrobacter* and 7 mg NH_3-N l^{-1} inhibited oxidation of ammonium to nitrite by *Nitrosomonas*. Therefore ammonia inhibition of nitrification in our systems is possible.
In order to confirm if nitrifiers are present in pond sediment and that DO is a limiting substrate, similar experiments of sediment and overlaying pond water at saturated DO maintained by continuous aeration for 5 days were carried out. For each system, three experiments (one during each experimental period) were conducted. Mixed samples of sediment from the 4 ponds were incubated at 20^{o}C and under DO saturated condition. Potential nitrification rates of 1150 (±43) and 890 (±14) mg-N m^{-2} d^{-1} in both samples

from algae and duckweed ponds were obtained, respectively. It could be concluded that nitrifiers were present in the sediment but their activities were suppressed at very low DO concentration due to the importance of DO concentration for nitrification (Stenstrom and Poduska, 1980). The higher nitrification rates obtained in this experiment compared with nitrification rates in the water phase can be explained by the fact that significantly more nitrifiers were present in the sediment but were not active due to unfavourable environmental conditions. Due to the absence of aerobic conditions in the sediment layer, coupled nitrification and denitrification mechanism was not occurring in our ponds. This mechanism could account for considerable N-removal (65% of the nitrogen removal by both coupled and uncoupled denitrification) when measured in sediments of shallow lakes (Van Luijn, 1997). The presence of nitrifying bacteria in the sediment furthermore would provide evidence that sedimentation of suspended solids and attached nitrifiers is a mechanism by which nitrifiers are removed from the water phase causing limitation of nitrification in natural systems. However, no experiment was made to support the hypothesis of attachment of nitrifying bacteria on suspended solids and research is needed to confirm this hypothesis.

Effect of Duckweed Cover on Nitrification

The presence of a duckweed mat provided additional surface for the attachment of nitrifiers. Comparing rates in DBPs for the top and middle layer during period 2 shows an average increase with 25 % in the top layer. However, the high volume to surface/mat ratio and the intensive harvesting (4-5 days) reduced the possibility that nitrifying bacteria would thrive on plants' surface or root zone (Alaerts *et al*., 1996). Duckweed cover is expected to reduce the lethal effect of solar radiation on nitrifiers due to light absorption or reflection. However, the shading provided by duckweed did not favour nitrification in DBPs over ABPs. The absence or reduced algae biomass due to the shading provided by duckweed mat resulted in lower surface area for attachment. Also, the lower DO concentrations in DBPs may be responsible for lower nitrification rates in this system when compared with ABPs. It also could be concluded that the effect of UV light on nitrification in ABPs appeared to play a minor role in limiting nitrification. This finding is in contrast with Azov and Tregubova (1995), who found a lethal effect of light on nitrification process in waste stabilisation reservoir. The presence of high algae matter in ABPs provided light attenuation and more surfaces for nitrifiers to attach. The lower concentration of algae in the fourth algae pond (period 1 and 2) as indicated by the significantly lower suspended solids in this pond compared with previous ponds (data not shown) could allow for less attachment and hence limit nitrification.

Denitrification

Denitrification mainly occurred in the sediment rather than in the water column due to higher amounts of organic substances, denser colonization with denitrifiers (Chen *et al*., 1972; Terry and Nelson, 1975) and lower DO. Lower denitrification rates were measured at lower temperature due to the reduction in bacterial activities. Availability of biodegradable organic substances is essential for denitrification process to occur. Organic loading of 15 kg-BOD ha^{-2} d^{-1} was sufficient for denitrification to take place in our system (Figure 6). The maximum depth of active denitrification within the sediment layer varies depending on the penetration of nitrate (Christensen *et al*., 1990).

Measured denitrification rates in the water phase (5-45 mg-N m^{-2} d^{-1}) were in agreement with the rates of 10–50 mg-N m^{-2} d^{-1} observed by Körner and Vermaat (Körner and Vermaat, 1998). Denitrification rates in the sediment were in agreement with values reported in river sediments and submerged macrophytes: 470 mg-N m^{-2}.d^{-1} (Christensen *et al*., 1990); 30-4000 mg-N m^{-2} d^{-1} (Körner, 1997).

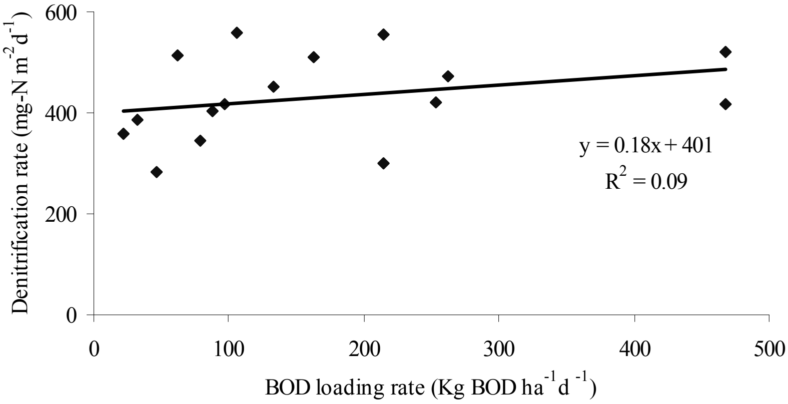


Figure 6. Relationship between denitrification rates and organic loading in both ABPs and DBPs during period 2 and 3.

The higher reported values in literature for submerged macrophytes could be explained by the availability of nitrite and nitrate in the water and the high surface area provided by the plants where denitrifiers could be attached. Negative net nitrification values in some ponds during period 3 suggest that denitrification potential of the ponds was greater than nitrification and that nitrification represents the bottleneck for nitrogen removal via denitrification. Denitrification corresponds to an average flux rate during the three periods of monitoring of 315, 517 and 538 mg-N m^{-2} d^{-1} in ABPs and 304, 371 and 487 mg-N m^{-2} d^{-1} in DBPs. These rates represent the following fractions of the total nitrogen input to the systems: 16, 25 and 24% in ABPs and 15, 18 and 22% in DBPs during the three periods of monitoring, respectively. Therefore denitrification provides an important nitrogen removal mechanism for both systems.
As nitrification is not increasing along the line of treatment, and is even decreasing in the fourth algae pond, increasing hydraulic retention time may not enhance nitrification rates and therefore neither denitrification. As stated before, surface for adsorption of nitrifiers is likely the most important limitation for nitrification in the pond water column. Introducing substrates for attachment in ponds for combined algae/bacteria biofilm growth may therefore increase nitrogen removal (Muttamara and Puetpaiboon, 1996).

CONCLUSIONS

The average nitrification rate from the 4 algae ponds lumped together was significantly higher than the average nitrification rate from the 4 duckweed ponds during the warm season operation, but similar rates were found in both systems at cold season operation. Nitrification rates of up to 200 mg-N m^{-2} d^{-1} were obtained at organic loading of 467 kg-BOD $ha^{-1}d^{-1}$ and higher rates (300-400 mg-N m^{-2} d^{-1}) were obtained at organic loading between 30-100 kg-BOD $ha^{-1}d^{-1}$. DO-nitrification rate relationship in the ABPs

and DBPs during the three experimental periods (cold temperature/low organic loading, warm temperature/low organic loading and warm temperature/high organic loading) followed Monod model with half saturation coefficient of 1.7 mg O_2 l^{-1}. Nitrification did not increase along the treatment line. It seems that the number of nitrifiers in the water phase did not increase in any of the ponds because nitrifiers that attached to disperse biomass or were present in suspension eventually settled to the anaerobic bottom where they would either die or become inactivated due to unfavourable conditions. Therefore, lack of surface for adsorption of nitrifiers in the pond water column is likely limiting for nitrifying microorganisms to remain in the water phase and for high nitrification rates to occur. Nitrifiers were present in the sediment but their activities were suppressed at very low DO concentration. The high volume to surface/duckweed mat ratio and the intensive harvesting reduced the possibility that nitrifying bacteria would thrive on plants' surface or root zone.
The average denitrification rate from the 4 algae ponds lumped together was significantly higher than rate from the 4 duckweed ponds during the low organic loading operation only. Denitrification corresponds to an average flux rate during the three experimental periods of 315, 517 and 538 mg-N m^{-2} d^{-1} in ABPs and 304, 371 and 487 mg-N m^{-2} d^{-1} in DBPs corresponded to 15-25% of total influent nitrogen. Therefore, denitrification provides an important nitrogen removal mechanism from both systems.

ACKNOWLEDGEMENTS

This work was financed by the Netherlands within the scope of the collaboration project "Water Sector Capacity Building in Palestine" between Birzeit University-West Bank and IHE-Delft-The Netherlands. The authors like to thank Arqam Hijawi and Nuha Ghonem (MSc. Students) for their valuable input in the practical work of this research. The authors are grateful to Dr. Henk Lubberding for critically reading the manuscript.

REFERENCES

Abeling U. and Seyfried C.F. (1992). Anaerobic-aerobic treatment of high-strength ammonia wastewater-nitrogen removal via nitrite. *Wat. Sci. Tech*. **26:** 1007-1015.

Alaerts G.J., Mahbubar M.R., Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res*. **30:** 843-852.

Andersen J.M. (1977). Rates of denitrification of undisturbed sediment from six lakes as a function of nitrate concentration, oxygen and temperature. *Arch. Hydrobiol*. **80:** 147-159.

APHA (1992). *Standard methods for examination of water and wastewater*. 18th edition, *Am. Publ. Assoc*., Washington D.C., USA.

Azov Y. and Tregubova T (1995). Nitrification processes in stabilisation reservoir. *Wat. Sci. Tech*. **31:** 313-319.

Belser W. and Mays E. L. (1982). Use of nitrifier activity measurements to estimate the efficiency of viable nitrifier counts in soil and sediments. *Appl. Environ. Microbiol*. **43:** 945-948.

Chen R.L., Keeney D.R., Graetz D.A. and Holding A.J. (1972). Denitrification and nitrate reduction in Wisconsin lake sediments. *J. Environ. Qual*. **1:** 158-162.

Christensen S., Simkins S. and Tiedje M. J. (1990). Spatial variation in denitrification: dependency of activity centres on the soil environment. *Soil Sci. Soc. Am. J*. **54:** 1608-1613.

Ferrara R. A. and Avci C. B. (1982). Nitrogen dynamics in waste stabilisation ponds. *J. WPCF*. **54:** 361-369.

Focht D.D. and Verstraete W. (1977). Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology*. **1:** 135-198.
Ginestet P., Audic J., Urbain V. and Block J. (1998). Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. *Appl. Environ. Microbiol*. **64:** 2266-2268.
Goeyens L., De Vries R.T.P., Bakker J.F. and Helder W. (1987). An experiment on the relative importance of denitrification, nitrate reduction and ammonification in coastal marine sediment. Neth. *J. Sea Res*. **21:** 171-175.
Green F.B., Bernstone L.S., Lundquist T.J. and Oswald W.J. (1996). Advanced integrated wastewater pond systems for nitrogen removal. *Wat. Sci. Tech*. **33:** 207-217.
Henriksen K. and Kemp W.M. (1988). Nitrification in estuarine and coastal marine sediments." In: Nitrogen cycling in coastal marine environments. Y.H. Blackburn and J. SØrensen (ed.), John Wiley and Sons Ltd., New York.
King D.L. (1978). The role of ponds in land treatment of wastewater. In: *Proc. International Symposium on Land Treatment of Wastewater*, Hanover, N.H. 191.
Körner S. (1997). Nutrient and Oxygen Balance of A Highly Polluted Treated Sewage Channel with Special Regard to the Submerged Macrophytes in Germany. *Acta hydrochim. Hydrobiol*. **3:** 34-40.
Körner S. and Vermaat J. E. (1998). The relative importance of *Lemna gibba* L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Wat. Res*. **2:** 3651-3661.
Metcalf and Eddy (1991). Wastewater engineering. Treatment, disposal and reuse. Tchobanoglous G. and Burton F. L. (ed.), McGraw – Hill. New York pp 700.
Muttamara S., Puetpaiboon U. (1996). Nitrogen removal in baffled waste stabilization ponds. *Wat Sci. Tech*. **33:** 173-181.
Pano A. and Middlebrooks E.J. (1982). Ammonia nitrogen removal in facultative wastewater stabilisation ponds. *J. WPCF*. **54:** 344-351.
Reed S.C. (1985). Nitrogen removal in stabilisation ponds. *J. WPCF*. **57:** 39-45.
Revsbech N.P., Nielson L.P., Christensen P.B. and Sørensen J. (1988). Combined oxygen and nitrous oxide microsensor for denitrification studies. *Appl. Environ. Microbiol*. **54:** 2245-2249.
Seitzinger S.P., Nixon S., Pilson M.E.Q. and Burke S. (1980). Denitrification and N_2O production in near-shore marine sediments. *Geochim. Cosmochim. Acta*. **44:** 1853-1860.
Silva S.A., de Oliveira R., Soares J., Mara D.D. and Pearson H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Wat. Sci. Tech*. **31:** 321-330.
Soares J., Silva S.A., de Oliveira R., Araujo A.L.C., Mara D.D. and Pearson H.W. (1996). Ammonia removal in a pilot-scale WSP complex in Northeast Brazil. *Wat. Sci. Tech*. **33:** 165-171.
Stenstrom M. K. and Poduska R. A. (1980). The effect of dissolved oxygen concentration on nitrification. *Wat. Res*. **14:** 643-649.
Taylor T. and Bishop P. (1989). Distribution and Role of Bacterial Nitrifying Population in Nitrogen Removal in Aquatic Treatment System. *Wat. Res*. **23:** 947-955.
Terry R.E. and Nelson D.W. (1975). Factors influencing nitrate transformations in sediments. *J. Environ. Qual.* **4:** 549.
Underhill S.G. and Prosser J.I. (1987). Surface attachment of nitrifying bacteria and their inhibition by potassium ethylxanthate. *Microbiol. Ecol*. **14:** 129-139.
Van Luijn F. (1997). Nitrogen removal by denitrification in the sediments of a shallow lake. Ph.D. Thesis. University of Wageningen, Wageningen, The Netherlands.
Verhagen F.J.M. and Laanbroek H.J. (1991). Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. *Appl. Env. Microbiol*. **57:** 3255-3263.
Vermaat J.E. and Hanif M.K. (1998). Performance of common duckweed species (*Lemnaceae*) and the Waterfern *Azolla Filiculoides* on different types of wastewater. *Wat. Res*. **32:** 2569-2576.
Verstraete W. and Alexander M. (1973). Heterotrophic nitrification in samples of natural ecosystems. *Environ. Sci. Technol.* **7**, 39-42.

Wiesmann U. (1994). Biological nitrogen removal from wastewater. *Adv. Bioch. Eng. Biotechnol*. **51:** 113-154.

Wong-Chong G.M. and Loehr R.C. (1978). The kinetics of Microbial Nitrification: Nitrite-Nitrogen Oxidation, *Wat. Res*. **12:** 605-609.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2000). Nitrogen transformation and wastewater treatment efficiency in algae-based and duckweed-based stabilisation ponds. In: *Proc. of the Conf. Managing Water and Waste in the New Millennium*.

Zimmo O.R., van der Steen N. P. and Gijzen H. J. (Submitted). Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilisation ponds.

Chapter 8

NITROGEN MASS BALANCE OVER PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTEWATER STABILISATION PONDS

Submitted as:

Zimmo O. R., van der Steen N. P. and Gijzen H. J. Nitrogen mass balance over pilot scale algae and duckweed-based wastewater stabilisation ponds. *Wat. Res*.

NITROGEN MASS BALANCE OVER PILOT-SCALE ALGAE AND DUCKWEED-BASED WASTEWATER STABILISATION PONDS

ABSTRACT

Nitrogen removal processes and nitrogen mass balances in algae based ponds (ABPs) and duckweed (*Lemna gibba*) based ponds (DBPs) were assessed during periods of four months, each under different operational conditions. During period 1 and 2 the effect of cold and warm temperature was studied. During period 2 and 3 the effect of low and high system organic loading (OL) was studied in warm seasons operation. The pilot-scale systems consisted of 4 similar ponds in series fed with domestic sewage with hydraulic retention time of 7 days in each pond. Overall nitrogen removal was higher during warm temperature in both ABPs and DBPs, but similar during period 2 and 3. Nitrogen removal in DBPs was lower than in ABPs by 20, 12 and 8% during cold temperature, warm temperature and high OL periods, respectively. Depending on temperature and OL rate, ABPs showed higher nitrogen removal via sedimentation (46-245% higher) compared to DBPs. Also, ABPs also showed higher nitrogen removal via denitrification (7-37% higher) compared to DBPs. Ammonia volatilisation in both systems did not exceed 1.1 % of influent total nitrogen during the entire experimental period. N-uptake by duckweed corresponds to 30% of the influent nitrogen during warm/low OL period and decreased to 10% and 19% during the cold and warm/high OL period, respectively. In ABPs, warm temperature resulted in significantly higher denitrification rates compared to cold temperature operation and denitrification increased with increase in OL. In DBPs, higher sedimentation and lower N-uptake rates were found during cold temperature compared to warm temperature operation. Denitrification and sedimentation were lower and N-uptake by duckweed was higher when comparing low and high OL periods.

Keywords: Algae ponds, duckweed ponds, *Lemna gibba*, nitrogen fluxes, nitrogen removal, wastewater treatment

INTRODUCTION

Nitrogen removal from wastewater has been subject of many studies during the last decade due to increasingly stringent environmental legislation in many countries. The uncontrolled release of nitrogen to the environment is known to cause serious pollution problems. Nitrate pollution in surface water and in groundwater has been attributed to wastewater outfalls and agricultural runoff. High nitrate levels in water can cause infant methaemoglobinaemia. Many rivers now contain >10 mg l^{-1} NO_3^--N and some occasionally exceed 50 mg l^{-1} NO_3^--N (Horne, 1995). Nitrogen as well as phosphorous plays a major role in eutrophication (Elser *et al*., 1990; Horne and Goldman, 1994). Besides, ammonia is toxic to aquatic organisms, especially the higher forms such as fish, at concentrations as low as 0.5 mgl^{-1} (Barnes and Bliss, 1983). Nitrogen in wastewater should be handled as a nutrient resource rather than a pollutant that only has to be disposed off (Gijzen and Mulder, 2001). Current mainstream technologies for wastewater treatment, such as the activated sludge process with N and P removal, are too costly to provide a satisfactory solution for the growing wastewater problems in developing regions (Gijzen and Ikramullah, 2001; Grau, 1994). The treatment of wastewater should be geared towards the effective re-use of nutrients (Gijzen and

Mulder, 2001). Waste stabilisation pond (WSP) systems are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants. However, N-removal in WSP has not been studied in great detail. Also, conventional WSP are not optimising nutrient reuse. Duckweed based pond system could be an attractive technology for wastewater treatment aiming at nutrient recovery and reuse. Additionally, because of the shading effect of duckweed cover, suspended solids (mainly algae) in the effluent will be much lower than for regular WSP (Zimmo *et al*., 2002). This would reduce algae problems, which represent one of the causes of emitter clogging in drip irrigation systems (Bucks *et al*., 1979).

On the basis of the low prevailing nitrate concentrations in pond systems several studies concluded that nitrification and denitrification do not play a major role in nitrogen removal (Ferrara and Avci, 1982; Mara and Pearson, 1986; Reed, 1985). Santos and Olivera (1987) and Zimmo *et al*., (Submitted b) however, demonstrated that nitrification is a major N-removal mechanism. In several studies ammonia volatilisation was proposed as a major mechanism for nitrogen removal from lagoons (Pano and Middlebrooks, 1982; Silva *et al*., 1995; Soares *et al*., 1996). Ferrara and Avci (1982) however claimed based on the same data that ammonia volatilisation accounts for only a small fraction of total N-removal and the main removal was via sedimentation and denitrification. Zimmo *et al*., (Submitted a) found that ammonia volatilisation in algae based ponds represents a minor fraction of nitrogen removal.

In duckweed systems, incomplete mass balances for nitrogen were made (Alaerts *et al.*, 1996; Boniardi *et al*., 1994; Körner and Vermaat, 1998; Vatta *et al*., 1995; Vermaat and Hanif, 1998; Zimmo *et al*., 2000a). These studies indicate that plant uptake of nitrogen is a major mechanism for nitrogen removal. Detrital settling usually accounts for less removal. Since the generally neutral pH does not support ammonia volatilisation, it was assumed that the nitrogen that could not be accounted for in these studies was due to denitrification. Nitrogen fixation is not expected to be important for the mass balance since it was previously observed that green algae (*Euglena* spec.) dominated the system (Zimmo *et al.* 2002).

So far no study has been undertaken to measure and compare the main nitrogen fluxes in ABP and DBP systems. In this study, nitrogen mass balances incorporating the main nitrogen fluxes were made under three different operational conditions.

MATERIALS AND METHODS

A pilot plant (Figure 1) with two parallel treatment systems of duckweed and algae based stabilisation ponds were continuously fed with domestic wastewater. The wastewater was received in a holding tank and subsequently pumped into the two parallel systems. Each system consisted of four ponds in series.

The residence time was 7days in each pond. Each pond had a depth of 0.9 m and a surface area of 3 m^2. Further details on the pilot system are presented elsewhere (Zimmo *et al*., 2000b). The systems were monitored during three periods of four months each, to study the effect of temperature and organic loading rate (OLR) on nitrogen transformations and removal mechanisms. Period 1 was from December 1999 to April 2000 during cold season (average water temperature=10±3 ^oC) and the influent wastewater was of low/medium strength (BOD=149±20 mg l^{-1}, n=8) according to Metcalf and Eddy (1991). Period 2 was from April to August 2000 during warm season (average water temperature=20±4 ^oC) using the same wastewater source as in period 1 (BOD=167±15 mg l^{-1}, n=8). Period 3 was from April to August 2001 during warm season (average water temperature=21±4 °C) and the wastewater used was of high organic load (BOD=375±32 mg l^{-1}, n=8) according to Metcalf and Eddy (1991).

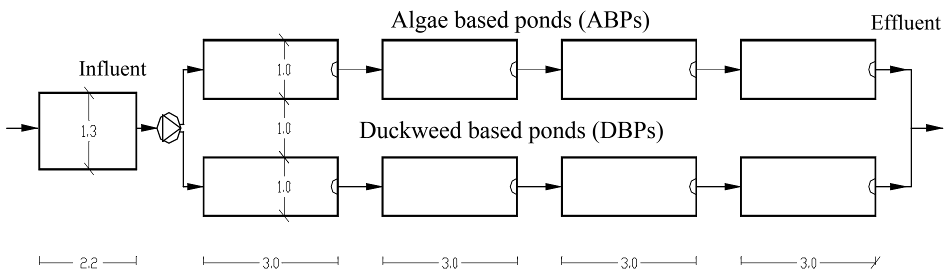


Figure 1. Schematic presentation of the stabilisation pond systems consisting of 4 algae and 4 duckweed based ponds (HRT=7 d each), preceded by a holding pond (HRT=1 d). Dimensions in meter.

Water balance

Inflow was kept at 0.38 m^3 d^{-1} in both ABPs and DBPs. Inflow was monitored by daily recording the water level in the holding tank. Clogging that might have occurred during one day was compensated by extra pumping the next day. Outflow of both systems was measured as an average of 2-hour interval measurements during two entire days for each experimental period. Outflow measurements during period 1 (rainy season) was done in two days without rain and was increased by average rainfall (480 mm) distributed evenly over period 1. Rainfall and weather temperature data were gathered from the meteorological station at Birzeit University at a distance of 500 m from the experimental site.

Nitrogen removal and nitrogen mass balance

Nitrogen removal from the ABP system and DBP system was described by the exponential relation: $N=N_I\ e^{-kt}$, (N_I: initial nitrogen concentration, N: nitrogen concentration at any time (t) along the line of treatment, *k*: removal coefficient).
The mass balance for nitrogen over ABPs system and DBPs system is given by Equation 1.

$$Q_{Inf}(N)_{Inf} = Q_{Eff}(N)_{Eff} + N_{Sed.} + N_{Dw} + N_{AVol} + N_{Denit.} + N_{unAcfor} \qquad (1)$$

where,

Q_{Inf} and Q_{Eff} = Inflow and outflow (l/d) through each system.
$(N)_{Inf}$ and $(N)_{Eff}$ = Influent and effluent nitrogen (Kj-N + NO_2^--N + NO_3^--N) concentrations (mg/l)
$N_{Sed.}$ = Nitrogen accumulation in the sediment (mg/d).
$N_{Dw.}$ = Nitrogen recovered (mg/d) via duckweed harvesting (for DBPs only).
$N_{AVol.}$ = Nitrogen leaving the system via ammonia volatilisation (mg/d).
$N_{Denit.}$ = Nitrogen leaving the system via denitrification (mg/d).
$N_{unAc\ for}$ = Unaccounted fraction of nitrogen (mg/d).

Influent and effluent nitrogen concentration of each pond was measured in grab samples collected at 10:00 hours once a week.

Duckweed and sediment sampling and measurements

Duckweed biomass was harvested every fifth day and duckweed density was restored to its initial density of 600 g fresh weight m^{-2}. Duckweed disappeared for about 25 days when water temperature dropped for a few days below 5^oC during January 1999 (period 1). During the rest of the period, duckweed was occasionally harvested. Nitrogen removal via biomass harvesting was determined by analysing triplicate samples of stored and dried duckweed for total nitrogen content. Duckweed relative growth rate (RGR) was calculated using the equation: RGR = (ln W2- ln W1)/t (Hunt, 1978), where W2 and W1 are the final and initial duckweed dry weights at each harvesting cycle period (t).
Sediment samples were collected at the beginning, middle and end of each experimental period by evacuating the sediment and overlaying water column inside a 2.3 cm glass tube immersed into the sediment layer. Triplicate samples were collected from each pond each time. Overlaying water was decanted and the N-content in the sediment was determined. Nitrogen accumulated in the sediment over each experimental period was calculated from the accumulated sediment every two month period.

Ammonia volatilisation and denitrification

Ammonia volatilisation was measured in all four ponds of both treatment systems (one pond each day) several times during each of the three experimental periods. Each measurement covered a 24 h period and was performed by pumping the air from an enclosed part of the pond surface and trapping the volatilised ammonia in a 90 ml 2% boric acid solution. Boric acid was then analysed for ammonium concentration. A detailed description of the methodology was reported elsewhere by Zimmo *et al.* (submitted a). Denitrification was measured using a nitrate reduction technique (Andersen, 1977). The number of denitrification measurements in each pond of the two systems was 7,4 and 4 during the three experimental periods, respectively. A detail description of the methodology is described elsewhere (Zimmo *et al.*, submitted b).

Analytical methods

The analytical methods for nitrogen compounds (Kjeldahl, ammonium nitrite and nitrate), BOD, temperature and pH were as described previously (Zimmo *et al.*, 2000b).

RESULTS

Average outflow was higher by 9% compared to average inflow during winter period and lower by 12 to 13% during the warm periods (Table 1). Slightly lower water loss (less than 2%) was measured in DBPs than ABPs probably due to the shielding provided by duckweed mat in the former system. Water flows to each pond in the two systems were corrected for the water loss or increase, which were later used in the nitrogen mass balance.

Table 1. Inflow (Q_{inf}), outflow (Q_{out}) and unaccounted for (UnAc for) as a percentage of inflow during the three experimental periods in ABPs and DBPs systems.

	Date	Q_{inf}	Water (T)	ABPs Q_{out}	ABPs Q_{avg}	ABPs UnAc for	DBPs Q_{out}	DBPs Q_{avg}	DBPs UnAc for
		m^3d^{-1}	^oC	m^3d^{-1}	m^3d^{-1}	% age	m^3d^{-1}	m^3d^{-1}	% age
Period 1	11/11/99	0.386	12.7	0.416*	0.419	-8.5	0.418*	0.421	-9.1
	11/01/00		6.5	0.422*			0.424*		
Period 2	4/11/00	0.386	18.4	0.336	0.336	12.9	0.34	0.3405	11.8
	6/11/00		21.3	0.336			0.341		
Period 3	4/11/01	0.386	18.6	0.339	0.337	12.8	0.346	0.3415	11.5
	6/11/01		20.8	0.334			0.337		

*Outflow was calculated as an average of 12 measurements done over entire day at 2 hour interval of no rain and was increased by average rainfall (480 mm) distributed evenly over period 1.

Influent and effluent (Kjeldahl and ammonium) nitrogen concentrations, N-uptake by duckweed, nitrogen accumulated in the sediments, nitrogen removed via ammonia volatilisation and via denitrification are shown in tables 2, 3, 4, 5 and 6, respectively. During the three experimental periods, no significant difference in nitrite and nitrate concentrations was observed between ABPs and DBPs. Nitrite did not exceed 3.1 mg-N l^{-1} and nitrate was less than 3.6 mg-N l^{-1}. Irrespective of experimental period, organic nitrogen in the final effluent of ABPs and DBPs varied from 6 to 9 and 1 to 3 mg l^{-1}, respectively. This corresponded to 10-15% and 2-4% of the influent nitrogen. Significantly higher removal rates in ABPs system (1079, 1448 and 1503 mg-N $m^{-2}d^{-1}$) were observed than for DBPs system (863, 1308 and 1380 mg-N $m^{-2}d^{-1}$) during the three experimental periods. Overall nitrogen removal was higher during warm temperature in both ABPs and DBPs, but similar during period 2 and 3. N-removal along the treatment line in ABPs and DBPs for the three experimental periods were best described by an exponential relation (R^2>0.98). *k*-values in the three experimental periods in ABPs (0.03, 0.05 and 0.04) were higher than in the DBPs (0.02, 0.03 and 0.03).

Table 2. Influent and effluent Kj-N and NH_4^+-N concentrations and total-N load in the ABPs and DBPs during the three experimental periods (n=15). Values are averages (±SD).

	Kj-N (mg l^{-1})			NH_4^+-N (mg l^{-1})			Total-N load (g-N/4 months)		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
Influent	61 (5)	61 (4)	69 (3)	60 (6)	59 (4)	62 (5)	2855	2847	3198
A1	51 (45)	44 (35)	53 (42)	45 (6)	34 (5)	42 (3)	2461	2102	2459
A2	39 (33)	32 (23)	41 (32)	33 (6)	23 (2)	31 (3)	1994	1537	1866
A3	31 (23)	23 (17)	31 (25)	23 (4)	17 (2)	24 (3)	1554	1076	1384
A4	25 (16)	15 (9)	24 (17)	14 (2)	9 (2)	17 (2)	1302	696	1033
D1	53 (3)	52 (3)	58 (3)	52 (6)	49 (2)	51 (4)	2545	2391	2669
D2	45 (4)	40 (2)	45 (5)	43 (4)	38 (2)	41 (4)	2236	1842	2063
D3	38 (5)	30 (2)	37 (5)	36 (3)	29 (2)	33 (6)	1934	1377	1630
D4	31 (4)	21 (2)	28 (5)	25 (4)	19 (1)	25 (5)	1613	963	1210

Table 3. Duckweed relative growth rates and N-uptake rates in DBPs during the three experimental periods. Values are averages (±SD).

	RGR			N-uptake rate (mg-N $m^{-2}d^{-1}$)		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
D1	0.05 (0.5)	0.12 (0.02)	0.04 (0.03)	180 (119)	461 (124)	122 (126)
D2	0.06 (0.06)	0.14 (0.02)	0.08 (0.05)	203 (99)	530 (66)	283 (168)
D3	0.06 (0.05)	0.14 (0.03)	0.13 (0.04)	204 (78)	602 (105)	551 (126)
D4	0.06 (0.05)	0.15 (0.03)	0.13 (0.04)	226 (93)	593 (143)	549 (124)
Average	0.06 (0.05)	0.14 (0.03)	0.09 (0.04)	203 (97)	547 (136)	376 (207)

Table 4. Nitrogen accumulation in the sediment in ABPs and DBPs during the three experimental periods.

	Pond	N-accumulation in sediment (g-N/4 months)				Pond	N-accumulation in sediment (g-N/4 months)		
		Period 1	Period 2	Period 3			Period 1	Period 2	Period 3
ABPs	A1	300	439	450	DBPs	D1	169	82	295
	A2	266	301	352		D2	127	101	293
	A3	209	187	269		D3	106	68	141
	A4	178	162	194		D4	113	64	138

Table 5. Ammonia volatilisation rates in ABPs and DBPs during the three experimental periods. Values are averages (±SD).

	Pond	Ammonia volatilisation rate (g-N/4 months)				Pond	Ammonia volatilisation rate (g-N/4 months)		
		Period 1	Period 2	Period 3			Period 1	Period 2	Period 3
ABPs	A1	4.9 (3.9)	8.5 (6.4)	6.4 (4.9)	DBPs	D1	3.8 (2.2)	6.5 (4.3)	6.4 (3.9)
	A2	4.5 (1.6)	10.5 (2.5)	10.0 (2.2)		D2	3.6 (2.5)	6.4 (2.0)	7.7 (1.4)
	A3	3.0 (1.1)	8.5 (2.4)	8.7 (2.3)		D3	3.6 (0.7)	4.2 (2.6)	5.9 (3.5)
	A4	1.4 (0.6)	4.9 (1.7)	5.4 (2.5)		D4	1.9 (0.6)	4.2 (1.7)	5.6 (1.9)

Table 6. Denitrification rates in ABPs and DBPs during the three experimental periods. Values are averages (±SD).

	Pond	Denitrification rate (g-N/4 months)				Pond	Denitrification rate (g-N/4 months)		
		Period 1	Period 2	Period 3			Period 1	Period 2	Period 3
ABPs	A1	90 (13)	200 (35)	188 (13)	DBPs	D1	74 (11)	109 (36)	151 (33)
	A2	118 (63)	201 (23)	170 (5)		D2	104 (14)	124 (25)	151 (15)
	A3	123 (60)	185 (15)	183 (10)		D3	90 (31)	139 (48)	163 (6)
	A4	57 (27)	101 (12)	150 (11)		D4	95 (58)	129 (22)	145 (7)

The various nitrogen fractions as a percentage of influent nitrogen for ABPs and DBPs during the three experimental periods are shown in Figure 2.

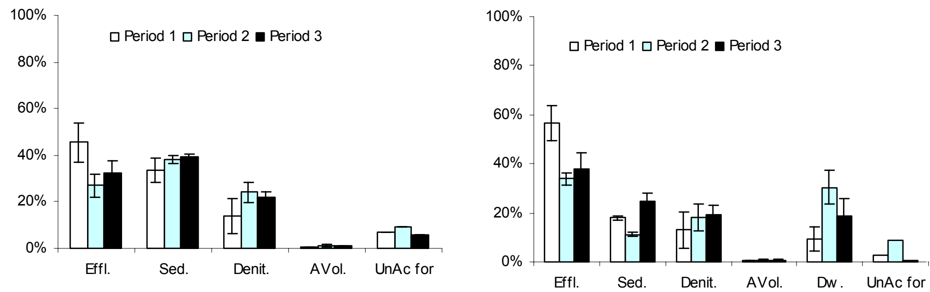


Figure 2. Various N-components in terms of percentages of the influent N for the three experimental periods. Error bars present SD.

In ABPs sedimentation and denitrification were the two main fluxes responsible for removing nitrogen from the wastewater. ABPs showed higher nitrogen removal via sedimentation (46-245% higher) compared to DBPs. The difference increased with higher temperature and lower OLR. Higher increase in sedimentation in ABPs over DBPs was observed during period 2. Denitrification and volatilisation rates were higher in ABPs than DBPs by 7-37% and 7-51%, respectively. Ammonia volatilisation during the experimental period presented only a small fraction of less than 1.2% of total influent nitrogen in both systems. In DBPs in addition to sedimentation and denitrification, duckweed harvesting was responsible for 10, 27 and 12% of the influent nitrogen during periods 1, 2 and 3, respectively. In ABPs, similar sedimentation and significantly higher denitrification were observed in period 2 when comparing with period 1. Comparing the same periods in DBPs, lower sedimentation and similar denitrification were observed. Significantly higher sedimentation was observed during period 3 in comparison with period 2 in both ABPs and DBPs.
The dominant algae species in ABPs was *Euglena* spec., therefore N-fixation by blue-green algae was considered negligible.

DISCUSSION

Overall nitrogen removal rates

There are no other comparative studies for ABPs and DBPs, but removal rate values observed in the two systems can be compared with values reported for individual systems in literature. Nitrogen removal during period 2 in ABPs is comparable to those of Silva (1982) who obtained 81% ammonium removal in a system with similar depth (1.0 m), influent Kjeldahl nitrogen of 54 mg l^{-1} and hydraulic retention time of 29 days, but operated at slightly higher ambient temperature (25 ^oC). Middlebrooks *et al*., (1982) reported higher removal values than observed in this study in systems with very long hydraulic retention times of 227 days. The nitrogen removal rate in the duckweed pond system (Table 2) was higher than values of 320 mg-N $m^{-2}d^{-1}$ reported by Alaerts *et al*. (1996), probably due to higher nitrogen concentration in the wastewater used in our study. Körner and Vermaat (1998) reported higher removal (73 to 97%) within 3 days in laboratory scale duckweed systems (18.5 cm diameter and 3.3 cm depth) most probably due to the high duckweed biomass per water volume ratio.

Effect of experimental variables on nitrogen fluxes in algae based ponds

The largest nitrogen flux in ABPs was sedimentation of particulate organic nitrogen, probably decaying algae biomass. The amount of algae growth is therefore an important factor, determining the amount of nitrogen accumulated in the sedimentation. An increase by 15% of nitrogen accumulated in sediment was observed during higher temperature (period 2) compared to lower temperature (period 1) probably due to more growth and corresponding algae decay at higher temperatures. The contribution of algae to sedimentation was similar during period 2 and 3. The importance of N-removal by sedimentation recognised in this study is consistent with Ferrara and Avci (1982), who found that sedimentation was the main removal pathway.
Denitrification behaved similarly when comparing the three experimental periods. Lower denitrification rates were measured at lower temperature due to the reduction in bacterial activities. During period 3 the increase of OL, especially in pond 1, did not result in lower overall system nitrification rate.
Despite the alkaline conditions in ABPs, ammonia volatilisation represented only a small fraction of nitrogen removal (less than 1.2% of total influent nitrogen). Comparing values during period 2 and 3, ammonia volatilisation seems not to be affected by increases in OL. As shown earlier, the pH, water temperature and ammonium concentration which affect volatilisation (Zimmo *et al.*, submitted a) were not significantly different during period 2 and period 3.

Effect of duckweed cover and experimental variables on the nitrogen fluxes

Nitrogen removal in DBPs was 20, 10 and 8% less than removal in ABPs during the three experimental periods, respectively. In DBP, the duckweed cover prevents sunlight penetration and consequently algae will not developed as in algae ponds. Therefore sedimentation was limited to the settlable suspended solids found in the wastewater and to the small portion of detritus duckweed plant that settled to the bottom of the ponds. The smaller amount of sediment produced in duckweed ponds in comparison with algae ponds will result in less frequent desludging requirements (Zimmo *et al.*, 2000b). Detrital sediment in DBPs was higher during period 1 and 3 due to the effect of low temperature (period 1) and high OLR (period 3), accounting for an increase of nitrogen content of the sludge by 63 % and 156%, respectively in comparison with period 2. Reduction in duckweed cover during period 1 and 3 allowed algae growth and settling of decayed algae that contributed to an increase in nitrogen accumulated in the sediment.
Nitrogen recovery via duckweed harvesting represented an important N-removal component. Duckweed growth was dramatically reduced when the water temperature dropped below 5 ^{o}C. BOD loading of 250 kg $ha^{-1}d^{-1}$ and higher, even at favourable water temperature, reduced N-uptake rates (Table 3). This was probably due to the slime layer developed on duckweed roots preventing nutrients uptake. Good correlation was found between average RGR over the harvesting cycles and both average ponds water temperature (during period 1 and 2) (R^2=0.84) and average pond water BOD loading rate (during period 2 and 3) (R^2=0.93).
Denitrification was identified as a significant flux for nitrogen removal rates in both ABPs and DBPs with DBPs having lower values than ABPs during period 2 only. This could be due to low nitrification, which is a limiting step for denitrification (Zimmo *et al.*, submitted b). As for ABPs, denitrification rate in DBPs was higher during the period of warm temperature.

Duckweed cover resulted in lower pH values, one of the main factors affecting ammonia volatilisation. Comparing the volatilisation rate in DBPs (n=114) with that in ABPs (n=114) showed significant higher NH_4^+-N removal rates in ABPs during the experimental periods. However, nitrogen removal via ammonia volatilisation during the three experimental periods in any system was minor and did not exceed 1.1 % of influent nitrogen.

Predictive model for N-removal

Each individual N-removal mechanism in each pond of the ABP system and DBP system is separately correlated with the experimental variables, which were measured during the experimental period and were found to affect the corresponding removal mechanism. Both N-removal rates (mg-N $m^{-2}d^{-1}$) by denitrification ($N_{denit.}$-rate) and sedimentation ($N_{sed.}$-rate) in the ABPs and the DBPs (calculated from Table 4 and Table 6) was fitted to the data of nitrogen loading rate ($\lambda_{s,N}$, kg-N $ha^{-1}d^{-1}$), organic loading rate ($\lambda_{s,BOD}$, kg-BOD $ha^{-1}d^{-1}$) (Data in Zimmo *et al.*, Submitted c) and temperature (T, ^oC) with multiple linear regression. Correlation coefficients (R^2) were 0.9 and 0.8 for ABPs and DBPs respectively ($p<0.01$). N-uptake by duckweed (Table 3) was found to correlate with temperature and organic loading rates (Data not shown). N-removal rate (mg-N $m^{-2}d^{-1}$) by duckweed uptake (N_{Dw}-rate) was also fitted with the same variables used to model N-removal by denitrification and sedimentation with multiple linear regression ($R^2 = 0.9$, $p < 0.01$). N-removal via ammonia volatilisation (mg-N $m^{-2}d^{-1}$) ($N_{AVol.}$-rate) was found to correlate with pond NH_3 concentration (mg l^{-1}) calculated on the basis of pond total ammonium concentration and pond pH value (Zimmo *et al.*, Submitted a). The results of the correlation between overall N-removal rate by different mechanisms and experimental variables are summarised in the model presented in equations 2 and 3.

For ABPs:

Overall N-removal rate = [$N_{denit.}$-rate + $N_{sed.}$-rate] + [$N_{AVol.}$-rate]

$$= [-317.8 + 15.5\ \lambda_{s,N} + 0.26\ \lambda_{s,BOD} + 2.87\ T] + [3.3\ NH_3 + 4.9] \quad (2)$$

For DBPs:

Overall N-removal rate = [$N_{sed.}$-rate + $N_{denit.}$-rate] + [$N_{AVol.}$-rate] + [N_{Dw}-rate]

$$= [978.2 - 10.1\ \lambda_{s,N} + 2.5\ \lambda_{s,BOD} + 4.8\ T] + [3.3\ NH_3 + 4.9] + [73.6 + 26.6\ \lambda_{s,N} - 1.2\ \lambda_{s,BOD} + 20.5\ T] \quad (3)$$

Good correlation between measured and predicted N-removal rates in both ABPs (R^2=0.95) and DBPs (R^2=0.85) is shown in Figure 3.

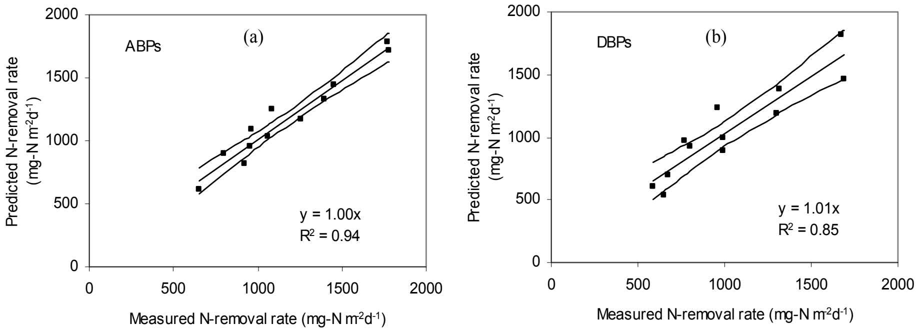


Figure 3. Measured N-removal rate versus predicted N-removal rate and the 95% confidence interval to the true mean value of N-removal in ABPs (a) and DBPs (b).

Model validation

With the absence of literature data for ponds operating under similar conditions, model validation was not possible. Using the model to predict effluent nitrogen in ponds with different design and/or operation conditions gave poor results. Actual nitrogen removal rates were lower on average by 28% compared to the predicted values when data were used from deeper pond depths (1.5 m) (Baldizon *et al*., 1995). Poor prediction (only 30%) of the actual nitrogen removal was found for waste stabilisation ponds of shallower depths (30-65 cm) as reported by Silva *et al*. (1995) and by van Haandel and Letting, 1994). It can be concluded therefore that pond depth probably plays an important role in nitrogen removal.

Small ponds depth of 30 to 65 cm results in high surface area per volume ratios. It is likely that this will enhance the surface and/or volume related processes of nitrogen removal, such as ammonia volatilisation, denitrification and sedimentation. In shallower ponds, the amount of light available per pond volume is higher compared to deeper ponds. This would result in higher algae growth and consequently in an increase in oxygen produced via photosynthesis. This would favour nitrification, the limiting step for denitrification and also nitrogen removal by sedimentation. The effect of pH, as a result of higher algae growth in shallower ponds, on nitrogen removal via volatilisation was taken into consideration in our model. In duckweed ponds, higher surface area per volume ratio will result in higher nitrogen removal via duckweed uptake (Körner and Vermaat, 1998; van der Steen *et al*., 1998).

The model as presented in this study is a first attempt to predict nitrogen removal from waste stabilisation ponds. Further elaboration and validation of the model to accommodate additional pond design and environmental parameters such as depth, temperature, intensity of solar radiation, algae growth etc., is required.

Unaccounted-for nitrogen in the mass balance

The nitrogen recovery measured for different nitrogen fluxes during the three experimental periods was approximately 91% of the nitrogen entering the systems. This means that the nitrogen budget in both ABPs and DBPs can be well explained with the nitrogen fluxes measured. Nitrogen that could not be accounted for could be attributed to the following reasons: a- statistical error in the measurements of nitrogen fluxes. b- nitrification and denitrification taking place in the biofilm attached to pond walls or duckweed roots and fronds. c- nitrogen uptake by mosquito larva, which eventually leave the system. This part of nitrogen was not quantified in our experiments and probably larger in ABPs.

CONCLUSIONS

Nitrogen removal in DBPs was lower by 20, 12 and 8% compared to removal in ABPs during cold temperature, warm temperature and high OL periods, respectively. The important nitrogen fluxes in ABPs were sedimentation and denitrification whereas harvesting of duckweed, denitrification and sedimentation were the major nitrogen fluxes in DBPs. Ammonia volatilisation in both systems did not exceed 1.1 % of influent total nitrogen in the treatment systems. ABPs showed higher nitrogen removal via sedimentation (46-245% higher) compared to DBPs. The difference increased with higher temperature and lower OLR. ABPs also showed higher nitrogen removal via denitrification (7-37% higher) and ammonia volatilisation (7-51% higher) compared to DBPs. The highest N-uptake by duckweed occurred during the warm and low OLR period. Predictive models for nitrogen removal presented a good reflection of nitrogen fluxes on overall nitrogen balance under the prevailing experimental conditions. Validation of the models for ABPs and DBPs with reported data from literature gave poor results for shallower ponds, while better agreement was obtained using data for deeper ponds. It was concluded that surface area per pond volume plays an important role in nitrogen removal from pond systems. The models as presented in this study are a first attempt to predict nitrogen removal from waste stabilisation systems. Further elaboration and validation of the model to accommodate pond design and environmental parameters that dictate pond performance is required.

ACKNOWLEDGEMENTS

The authors are grateful to the Dutch government for financially supporting this research within the collaboration project (WASCAPAL) between Birzeit University, West Bank and the UNESCO-IHE Institute for Waster Education, The Netherlands.

REFERENCES

Alaerts G.J., Mahbubar M.R., Kelderman P. (1996). Performance of a full-scale duckweed-covered sewage lagoon. *Wat. Res*. **30:** 843-852.

Andersen J.M. (1977). Rates of denitrification of undisturbed sediment from six lakes as a function of nitrate concentration, oxygen and temperature. *Arch. Hydrobiol*. **80:** 147-159.

Baldizon M.E., Dolmus R., Quintana J., Navarro Y. and Donze M. (2002). Comparison of conventional and macrophyte-based systems for the treatment of domestic wastewater. *Wat. Sci. Tech*. **45**(1): 111-116.

Barnes D. and Bliss P.J. (1983). Biological control of nitrogen in wastewater treatment. E and F.N. Spon, London, UK. 3 pp.

Boniardi N., Vatta G., Rota R., Nano G. and Carra S. (1994). Removal of water pollutants by *Lemna gibba. Chem. Eng. J*. **54:** 41-48.

Bucks D. A., Nakayama F.S. and Gilbert R.G. (1979). Trickle water quality and preventive maintenance. *Agricultural Water Management*. **2:** 149-162.

Elser J.J, Marzolf E.R. and Goldman C.R. (1990). Phosphorus and nitrogen limitation of phytoplankton in the freshwaters of North America: a review of critique of experimental enrichments. *Can. J. Fish. Aquat. Sci*. **47:**1468-1477.

Ferrara R. A. and Avci C. B. (1982). Nitrogen dynamics in waste stabilisation ponds. *J.WPCF*. **54**(4): 361-369.

Gijzen H.J. and Ikramullah M (1999). Pre-feasibility of duckweed-based wastewater treatment and resource recovery in Bangladesh. *World Bank Report*, Washington D.C., pp194.

Gijzen H.J. and Mulder A. (2001). The nitrogen cycle out of balance. *Water21*, August 2001, pp. 38-40.

Grau, P. (1994). What's next? *Wat. Qual. Intl*. **4:** 29-32.

Horne A.J. (1995). Nitrogen removal from waste treatment pond or activated sludge plant effluents with free-surface wetlands. *Wat. Sci. Tech*. **31:** 341-351.

Horne A.J. and Goldman C.R. (1994). *Limnology*. McGraw-Hill, New York. Second Edition, 627 pp.

Hunt R. (1978). Plant growth analysis. Edward Arnold, London. 67 pp.

Körner S. and Vermaat J. E. (1998). The relative importance of Lemna gibba L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. *Wat. Res.* **32:** 3651-3661.

Mara D.D. and Pearson H.W. (1986). Artificial fresh water environments: waste stabilisation ponds. In *Biotechnology*, Ed. W. Schoernborn, pp. 177-206.

Metcalf and Eddy (1991). Wastewater engineering. Treatment, disposal and reuse. Tchobanoglous G. and Burton F. L. [eds.], McGraw – Hill. New York.

Middlebrooks, E.J., Middlebrooks C.H., Reynolds J.H., Watters G.Z., Reed S.C. and George D.B. (1982). Wastewater stabilization lagoon design, performance and upgrading. Macmillan, NY, USA.

Pano A. and Middlebrooks E.J. (1982). Ammonia nitrogen removal in facultative wastewater stabilisation ponds. *J.WPCF.* **54:** 344-351.

Reed S.C. (1985). Nitrogen removal in stabilisation ponds. *J.WPCF*. **57**(1): 39-45.

Santos M.C.R. and Oliveira J.F.S. (1987). Nitrogen transformations and removal in waste stabilisation ponds in Portugal. *Wat. Sci. Tech*. **19**(12): 123-130.

Silva S. A. (1982). On the treatment of domestic sewage in waste stabilisation ponds in N.E. Brazil. PhD thesis, University of Dundee, UK.

Silva S.A., de Pliveira R., Soares J., Mara D.D. and Pearson H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Wat. Sci. Tech*. **31:** 321-330.

Soares J., Silva S.A., de Oliveira R., Araujo A.L.C., Mara D.D. and Pearson H.W. (1996). Ammonia removal in a pilot-scale WSP complex in Northeast Brazil. *Wat. Sci. Tech*. **33:** 165-171.

van der Steen P., Brenner A. and Oron G. (1998). An integrated duckweed and algae pond system for nitrogen removal and renovation. *Wat. Sci. Tech*. **38**: 335-343.

van Haandel A.C. and Lettinga G. (1994). Anaerobic sewage treatment. A practical guide for regions with hot climate. Jon Wiley and Sons.

Vatta G., Rota R., Boniardi N. and Nano G. (1995). Dynamic modeling of wastewater treatment plants based on *Lemna gibba. Biochemical Engineering Journal.* **57:** 37-48.

Vermaat J.E. and Hanif M.K. (1998). Performance of Common Duckweed Species (*Lemnaceae*) And the *Waterfern Azolla Filiculoides* on Different Types of Wastewater. *Wat. Res.* **32**(9): 2569-2576.

Wat . Sci. Tech. **31:** 21-31.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2000a). Comparison between algae-based and duckweed-based wastewater treatment: differences in environmental conditions and nitrogen transformations. *Wat. Sci. Tech*. **42**(10): 215-222.

Zimmo O. R., Al-Sa'ed R., van der Steen P. and Gijzen H. J. (2000b). Nitrogen transformation and treatment efficiency in algae-based and duckweed-based stabilisation ponds. *Conference proceeding "Managing Water and Waste in the New Millennium"*.

Zimmo O.R., Al-Sa`ed R. M., van der Steen N. P. and Gijzen H. J. (2002). Process performance assessment of algae-based and duckweed-based wastewater treatment systems. *Wat. Sci. Tech*. **45:** 91-101.

Zimmo O. R., van der Steen N. P. and Gijzen H. J. (Submitted, a). Comparison of ammonia volatilisation in algae and duckweed-based waste stabilisation ponds.

Zimmo O. R., van der Steen N. P. and Gijzen H. J. (Submitted, b). Quantification of nitrification and denitrification rates in algae and duckweed-based waste stabilisation ponds.

Zimmo O. R., van der Steen N. P. and Gijzen H. J. (Submitted, c). Effect of organic surface load on process performance of pilot-scale algae and duckweed-based waste stabilisation ponds.

SUMMARY

Nitrogen in wastewater is an important pollutant causing eutrophication in surface water and causes ground water pollution by nitrate. In recognition of the deteriorating effects of nitrogen on water resources, standards for effluent discharges are becoming stricter, and nutrient removal becomes one of the prerequisites for selection of wastewater treatment systems.

There are a variety of technologies available to reduce organic compounds and nitrogen levels in municipal wastewater. Current wastewater treatment technologies such as activated sludge systems, with tertiary nitrogen removal, are too costly to provide a satisfactory solution for the increasing wastewater problems in developing regions. These technologies do not allow for reuse of valuable energy, water and nutrients contained in wastewater. Wastewater treatment technologies that aim at energy recovery, nutrient recovery, water reuse and pollution prevention should be considered since these may be attractive and affordable world-wide. Conventional waste stabilisation ponds, in this research referred to as algae-based ponds (ABPs) to emphasise the role of algae in such treatment systems, provide effluent of good quality for safe reuse in agriculture. However, nitrogen removal and utilisation is not optimised in this system. The effluent contains algae biomass and upon degradation nitrogen is released into the environment. Duckweed-based ponds (DBPs) are modified waste stabilisation ponds covered with floating aquatic plants. These systems are low cost and do not need sophisticated equipment, high energy or qualified labour input. DBPs have the potential for effective nitrogen recovery from wastewater in the form of duckweed protein and therefore less nitrogen removed via other mechanisms.

Nitrogen removal in the context of this thesis is defined as the difference between influent and effluent nitrogen. This is caused by different removal mechanisms such as sedimentation, denitrification, volatilisation and duckweed uptake.

Nitrogen recovery in the context of this thesis is defined as the amount of nitrogen that is removed from wastewater in such a way that it could potentially be reused as a valuable nitrogen source. Nitrogen recovery therefore can be realised by duckweed harvesting (animal feed), sedimentation (fertiliser) and via effluent reuse for irrigation (plant uptake of remaining nitrogen).

So far, limited information is available on the fate of nitrogen in algae-based ponds and even less information is available for duckweed-based ponds. This PhD thesis quantifies the individual nitrogen removal mechanisms under different seasonal variations and different strengths of wastewater. The thesis compares the behaviour of nitrogen in algae and duckweed-based ponds with a view to identify effective strategies for optimal nitrogen recovery. The methodology adopted in the study includes short-term batch outdoor experiments and long-term pilot-scale experiments.

Batch experiments simulating algae and duckweed (*Lemna gibba*) based waste stabilisation ponds were monitored over periods of 15 days to determine the effect of pH and DO on nitrogen removal mechanisms in both systems (**Chapter 2**). In both systems, N-removal by different mechanisms are more dependent on the pH variations than on the oxygen variations. At high pH ranges (7-9) under both anaerobic and aerobic conditions, N-removal was significantly higher in comparison with low pH ranges (5-7) in both systems due to an increase in both sedimentation and ammonia volatilisation. Significantly higher N-removal efficiencies in the duckweed system (26-33%) than in the algae system (14-24%) was found at lower pH range of 5-7. The

principal N-removal mechanisms in duckweed system at low pH were N-uptake by duckweed and N-removal via uptake by biomass attached to container wall and in the sediment. The increase in N-removal by sedimentation and volatilisation in the algae system and the decrease in N-uptake by duckweed in the duckweed system at high pH values (7-9) resulted in significantly higher N-removal efficiencies in the algae system (45-60%) than the duckweed system (38-41%).
N-removal in the algae incubation without oxygen control at high pH range (7-9) was 17-20% higher compared to the anaerobic and aerated incubations, due to higher nitrification/denitrification and sedimentation. In duckweed systems this was 22-25% higher.

Further comparison between algae and duckweed-based stabilisation ponds was carried out in continuous flow pilot-scale pond systems. Each system consisted of 4 equal ponds in series fed with wastewater with hydraulic retention time of 7 days in each pond. The difference in the hydraulic characteristics (**Chapter 3**) was studied by conducting a tracer experiment using LiCl. Results showed that the hydraulic characteristics were very similar in ABPs and DBPs. Actual retention times were observed to be higher than the theoretical retention times due to the spurious tracer curves resulting in negative dead spaces. We concluded that probably the LiCl, because of its higher density compared to wastewater, passed onto the bottom of the pond and slowly leached out over a much longer period. The tracer's peak was detected at the outlets of various ponds at 74-96% of the theoretical retention times indicated limited short-circuiting. The hydraulic nature of the ponds was neither plug-flow nor completely mixed but dispersed flow. A tracer experiment on a larger scale algae and duckweed pond at Ginebra-Colombia showed lesser short-circuiting and more plug flow conditions in the duckweed pond than the algae pond. The better hydraulic behaviour of the pond with duckweed cover may be explained by reduced wind-induced short circuiting and reduced mixing caused by the absence of algae biomass.

The effect of seasonal variation in the environmental characteristics and process performance of algae and duckweed-based waste stabilisation ponds is described in **Chapter 4**. The growth of algae in ABPs resulted in higher (day time) values for pH and dissolved oxygen due to algae photosynthesis; in DBPs values were lower as a result of shading provided by the duckweed cover. The higher intensity of solar radiation, pH and dissolved oxygen in algae ponds stimulated the natural decay of faecal coliforms (FC) compared to duckweed ponds. Higher BOD and TSS removal efficiencies were achieved in DBPs compared to ABPs. Total-P was more effectively reduced in DBPs than in ABPs, irrespective of the season due to phosphorus uptake by duckweed and subsequent removal from the system via harvesting. During the summer period the average total nitrogen was reduced more in ABPs (80%) than in DBPs (55%), despite the fact that approximately one third of the influent nitrogen to the DBPs was removed via duckweed harvesting. Lower removal efficiencies for nitrogen in both systems were obtained during the winter season. In DBPs 33% and 15% of the total nitrogen was recovered into biomass and removed from the system via duckweed harvesting during the summer and winter period respectively.

The effect of organic loading on removal efficiency of ABPs and DBPs was investigated and reported in **Chapter 5**. BOD and TSS removal rates increased with increase in organic loading for both systems. During both experimental periods of low and high organic loading, higher BOD and TSS removal efficiencies were found in the duckweed system as compared to the algae system. Similar overall N-removal rate was found during low and high organic loading periods in both systems. Overall N-removal rate in ABPs was significantly higher than that in DBPs. N-uptake by duckweed in duckweed system was 30% and 19% of the total nitrogen input during the low and high organic loading periods, respectively. Organic loading had no effect on total phosphorus removal in both ABPs and DBPs. Higher P-removal was achieved in DBPs than ABPs. In both periods, FC removal was higher in ABPs system than in DBPs system. Faecal coliforms removal for ABPs in low organic loading period and high organic loading period was 3.8 and 3.4 log units, respectively. In DBPs 2.2 and 1.76 log removal was observed for the two periods, respectively.

In literature contradictory conclusions were drawn on the role of ammonia volatilisation from waste stabilisation ponds. In contrast with previous studies, in which only initial and final concentrations of nitrogen were considered to predict ammonia volatilisation, the technique followed here allowed actual measurements of the nitrogen flux via this mechanism (**Chapter 6**). In this study a description of the development and application of a simple method for direct assessment of ammonia volatilisation rate from waste stabilisation ponds was elaborated. The method developed can also be used to assess the quantitative contribution of treatment systems to other gas emissions with potential negative environmental impact (e.g. CH_4, NO_x, H_2S)**.** Ammonia volatilisation was assessed in a continuous flow pilot plant during a period of one and half year. Ammonia volatilisation rates in the ABPs were higher than in the DBPs. The volatilisation rates correlated well with free ammonia concentrations (NH_3) in pond water. Lower values of NH_3 in DBPs due to lower pH resulted in lower ammonia volatilisation rates in comparison with ABPs. Whenever ammonia concentrations were the same for any of the algae or duckweed ponds, also ammonia volatilisation rates were similar. Apparently, the duckweed cover did not physically hinder ammonia volatilisation. Volatilisation was in the range of 7.2 to 37.4 mg-N $m^{-2}d^{-1}$ and 6.4 to 31.5 mg-N $m^{-2}d^{-1}$ in ABPs and DBPs, respectively. The significance of ammonia volatilisation in the pilot-scale pond systems was limited to less than 1.1 % of total influent nitrogen to the treatment systems.

The differences in the conditions in pond systems due to the presence and/or absence of a duckweed cover triggered differences in nitrification and denitrification rates (**Chapter 7**). Higher DO concentrations in ABPs, especially during the warm season, favoured higher nitrification in ABPs over DBPs. Nitrification rates did not increase along the treatment line despite the decrease in organic matter content and corresponding increase in DO. In both systems denitrification (rates: 160-560 mg-N $m^{-2}d^{-1}$) was mainly observed in the sediments where anaerobic conditions prevailed. Nitrification and denitrification rates in both systems were higher at high temperature (21 ^{o}C) compared to low temperature (11 ^{o}C). The range of nitrogen removal via denitrification in ABPs and DBPs corresponded to 15-25% of total influent nitrogen.

With the nitrogen removal rates due to different removal mechanisms gathered in the previous chapters, nitrogen mass balances were established in both algae and duckweed-based ponds (**Chapter 8)**. Irrespective of organic loading condition and season, the major fluxes of nitrogen in the ABPs were sedimentation and to a lesser extend denitrification. In DBPs, sedimentation and denitrification were of equal importance except during the warm season and low organic loading when sedimentation was low. Nitrogen removal via harvested duckweed biomass was also important especially during warm season and low organic loading. Ammonia volatilisation in both systems did not exceed 1.1 % of total nitrogen removal during the entire experimental period. Algae and duckweed incubations conducted in chapter 2 revealed higher ammonia volatilisation rates compared with resulted obtained in this chapter using pilot-scale systems. Shallow water depth and higher pH values that were maintained in batch incubations favoured high undissociated NH_3 and therefore higher volatilisation rate. Predictive models for nitrogen removal in ABPs and DBPs were proposed. They presented a good reflection of nitrogen fluxes on overall nitrogen balance under the prevailing experimental conditions. Validation of the models with reported data from literature gave poor results for shallower ponds, while better agreement was obtained using data for deeper ponds. Further elaboration and validation of the model to accommodate pond design and environmental parameters that dictate pond performance is required.

Consequences for design and operation of algae and duckweed-based stabilisation ponds

Two different treatment objectives could be defined to manage nitrogen in ABPs and DBPs. The first would be to maximise nitrogen removal to protect the receiving water resources from eutrophication. Another objective could be to maximise nitrogen recovery for subsequent reuse. Considering the sustainability concepts for wastewater management as discussed earlier, wastewater treatment technology should aim at maximising nitrogen recovery.

In this study, it could be concluded that duckweed-based pond system allows for nitrogen recovery (via duckweed uptake, sedimentation and effluent irrigation) which has a high value for a range of reuse options in both aquaculture and agriculture. Effluent from DBPs presents less problems in (drip) irrigation due to lower concentration of suspended solids compared to the effluent from ABPs. The feasibility of DBPs is determined not only by the process performance but also by the simplicity of the system and economic benefits of by products, such as duckweed biomass.

A reduction of nitrogen from 60 to 30 mg-N l^{-1} under the conditions of this work (21 ^{o}C) requires a HRT of 24 and 30 days in ABPs and DBPs of 0.9 m depth. To achieve the same reduction of nitrogen, the area of DBPs should be enlarged by 10% during periods of high organic loading to 25% during cold season. This will cause extra land requirements and costs but it also generates income from duckweed production and reuse.

The design of ABPs could be adjusted to maximise sedimentation and minimise denitrification (the main nitrogen removal mechanisms) in order to maximise nitrogen recovery. Better N removal by sedimentation requires a combination of enhancing N-uptake by algae and enhanced decay and settling. Increasing pond surface area by reducing pond depth is expected to increase algae growth, as in high rate algae ponds, but this will also increase land costs. Providing a duckweed pond that receives algae pond effluent, where sunlight is shielded to facilitate algae settling, may increase overall nitrogen recovery through sedimentation of decayed algae and via duckweed uptake. Decreasing pond depth could lead to an increase in nitrogen removal via ammonia volatilisation, which is environmentally unfavourable. Therefore more studies are needed to optimise pond depth in order to favour sedimentation and to reduce ammonia volatilisation.

Nitrogen recovery by duckweed could be optimised by maintaining favourable conditions for duckweed growth. This could be achieved via designing ponds at appropriate organic and nitrogen loading. Optimisation of duckweed growth in terms of species selection, stocking density, harvesting regime, pond geometry should be further investigated.

The second major mechanism for nitrogen removal in algae and duckweed ponds is nitrification and denitrification. If the water column is kept aerobic including the top layer of the sediment where the presence of nitrifiers was evident, nitrification could be maximised and denitrification could be minimised. Introducing media into the pond to provide more surface area for attachment of nitrifiers is a possible alternative for enhancing nitrification.

SAMENVATTING

Stikstof omzettingen en verwijderingsmechanismen in algen en eendekroos-vijvers voor stabilisatie van afvalwater

Stikstof in afvalwater kan eutrofiëring van oppervlaktewater veroorzaken, evenals de vervuiling van grondwater met nitraat. Vanwege deze negatieve effecten van stikstof op de waterkwaliteit worden de lozingseisen voor effluent steeds stringenter en nutriëntenverwijdering is daarmee één van de selectiecriteria voor afvalwaterzuiveringssystemen geworden. Er is een verscheidenheid aan technologieën beschikbaar om vervuiling door organische stof en stikstof in gemeentelijk rioolwater te reduceren. De huidige technologieën voor afvalwaterzuivering, zoals het actiefslib systeem waarmee stikstofverwijdering mogelijk is, zijn te duur om een goede oplossing voor het groeiende probleem van afvalwater in ontwikkelingslanden te bieden. Deze technologieën zijn namelijk niet gericht op het hergebruik van waardevolle energie, water en nutriënten die in het afvalwater aanwezig zijn. Technologieën voor afvalwaterzuivering die gericht zijn op terugwinning van energie, terugwinning van nutriënten, hergebruik van water en het voorkómen van vervuiling moeten overwogen worden omdat deze technologieën aantrekkelijk en betaalbaar zijn. Conventionele vijvers voor stabilisatie van afvalwater, in dit onderzoek algen-vijvers (ABPs) genoemd om de rol van algen in dergelijke systemen te benadrukken, leveren een goede kwaliteit effluent voor veilig hergebruik in de landbouw. Echter, stikstofverwijdering en terugwinning zijn niet optimaal in dat systeem. Het effluent bevat namelijk algen-biomassa en na afbraak hiervan komt de stikstof weer vrij in het milieu. Eendekroos-vijvers (DBPs) zijn aangepaste stabilisatievijvers. Ze zijn bedekt met een laag drijvende waterplanten. Deze systemen zijn goedkoop en hebben geen ingewikkelde apparatuur nodig, geen hoog energieverbruik en geen inzet van gekwalificeerd personeel. Met behulp van DBPs kan effectief stikstof terug gewonnen worden uit afvalwater in de vorm van eendekroos-eiwit en zo gaat er minder stikstof verloren door andere mechanismen.
Stikstof verwijdering in de context van dit proefschrift is gedefinieerd als het verschil tussen de stikstof aanwezig in het influent en effluent. Verwijdering wordt veroorzaakt door verscheidene mechanismen, zoals sedimentatie, denitrificatie, vervluchtiging en opname door het eendekroos. Stikstof terugwinning is in dit proefschrift gedefinieerd als de hoeveelheid stikstof die verwijderd is uit het afvalwater op een dergelijke wijze dat het potentieel hergebruikt kan worden als waardevolle stikstofbron. Stikstof terugwinning kan daarom gerealiseerd worden door oogsten (dierenvoeder), sedimentatie (meststof) en via effluent hergebruik voor irrigatie (opname van stikstof door planten).

Tot op heden is maar beperkte informatie beschikbaar over het lot van stikstof in algen-vijvers en nog minder informatie is beschikbaar voor eendekroos-vijvers. In dit proefschrift worden de verscheidene stikstof verwijderingsmechanismen gekwantificeerd tijdens de verschillende seizoenen en tijdens de belasting met afvalwaters van verschillende concentraties aan vervuilende stoffen. In dit proefschrift wordt het gedrag van stikstof in algen-vijvers en eendekroos-vijvers vergeleken met als doel om effectieve strategieën voor optimale stikstofterugwinning te bepalen. De methodologie die daarvoor is gebruikt omvat kort durende batch experimenten in de open lucht en langdurige experimenten met een proefinstallatie (pilot-scale).

Batch experimenten voor het simuleren van algen en eendekroos (*Lemna gibba*) vijvers werden bemonsterd gedurende 15 daagse perioden om het effect van pH en DO (concentratie opgeloste zuurstof) op stikstof verwijderingsmechanismen te bepalen in beide systemen **(Hoofdstuk 2)**. In beide systemen is stikstofverwijdering door de verschillende mechanismen meer afhankelijk van de pH variaties dan van de DO variaties. Bij hoge pH waarden (7-9) onder zowel aërobe als anaërobe condities was stikstofverwijdering significant hoger dan bij lage pH waarden (5-7) in beide systemen door een toename van zowel sedimentatie als ammoniak vervluchtiging. Bij lagere pH waarden (5-7) werd significant hogere stikstof verwijdering in het eenden-kroos systeem (26-33%) dan in het algen-systeem (14-24%) waargenomen. De belangrijkste stikstof verwijderingsmechanismen in het eendekroos-systeem bij lage pH waren de opname van stikstof door eendekroos en opname door biomassa die groeide op de wand van de bekers en in het sediment. De toename in stikstof verwijdering door sedimentatie en vervluchtiging in het algen-systeem en de afname van opname van stikstof door eendekroos in het eendekroos-systeem bij hoge pH waarden (7-9) resulteerde in significant hogere stikstof verwijdering in het algen-systeem (45-60%) in vergelijking met het eendekroos-systeem (38-41%). Stikstof verwijdering in de algen incubatie zonder controle van de zuurstof concentratie bij hoge pH waarden (7-9) was 17-20% hoger in vergelijking met de anaërobe en de beluchte incubaties, vanwege hogere nitrificatie/denitrificatie en sedimentatie. In eendekroos-systemen was dit 22-25% hoger.

Verdere vergelijking van algen- en eendekroos vijvers is uitgevoerd in een continu bedreven proefinstallatie van vijvers. Elk systeem bestond uit 4 gelijke vijvers in serie, zodanig gevoed met afvalwater dat de hydraulische verblijftijd in elke vijver 7 dagen was. Het verschil in hydraulische eigenschappen (**Hoofdstuk 3**) is bestudeerd door het uitvoeren van een tracer experiment met LiCl. De resultaten lieten zien dat de hydraulische eigenschappen in ABPs en DBPs sterk overeen kwamen. De waarnemingen lieten zien dat werkelijke verblijftijden langer waren dan de theoretische verblijftijden, met als gevolg dat een negatieve dode hoek berekend werd. Wij concludeerden dat het LiCl waarschijnlijk naar de bodem zonk, door de hogere dichtheid van een LiCl oplossing in vergelijking met afvalwater. De tracer is vervolgens weer langzaam uit de dode hoek gelekt over een lange periode. De piek in tracer concentratie werd gedetecteerd aan de vijver uitlaat van de verschillende vijvers na 74-96% van de theoretische verblijftijd en dit gaf aan dat kortsluitstromen maar beperkt optraden. Het hydraulisch karakter van de vijvers was niet als een propstroom noch als een volledig gemengd systeem, maar werd het best beschreven door dispersie-stroming. Een tracer experiment in meer grootschalige algen- en eendekroos-vijvers in Ginebra (Colombia) liet minder kortstromen en meer propstroom condities in de eendekroos-vijver dan in de algen-vijver zien. De betere hydraulische karakteristiek van eendekroos-vijvers kan verklaard worden door een afname van kortsluitstromen veroorzaakt door wind en afname van menging veroorzaakt door de aanwezigheid van algen.

Het effect van seizoensinvloeden en de milieu omstandigheden in de vijvers op het functioneren van de algen en eendekroos-vijvers is beschreven in **Hoofdstuk 4**. De groei van algen in ABPs resulteerde overdag in hogere waarden voor pH en zuurstofconcentratie vanwege algen fotosynthese. In DBPs waren de waarden lager als gevolg van schaduw veroorzaakt door de eendekroos-bedekking. De hogere intensiteit zonlicht, pH en zuurstofconcentratie in algen-vijvers stimuleerde de natuurlijke afsterving van fecale coliformen (FC) in vergelijking met eendekroos-vijvers. Hogere

BZV en TSS verwijdering werden gerealiseerd in DBPs in vergelijking met ABPs. Totaal-P werd effectiever verminderd in DBPs dan in ABPs., onafhankelijk van het seizoen, vanwege opname van fosfor door eendekroos en de verwijdering uit het systeem door middel van oogsten. Tijdens de zomerperiode was de gemiddelde verwijdering van stikstof in ABPs (80%) hoger dan in DBPs (55%), ondanks dat in DBPs ongeveer een derde van de stikstof uit het influent verwijderd werd via het oogsten van eendekroos. Lagere verwijderingsefficiënties voor stikstof werden verkregen in beide systemen tijdens de winter. In DBPs werd in de zomer 33%en in de winter 15% van de totale hoeveelheid stikstof terugwonnen in de biomassa en verwijderd uit het systeem door het oogsten van eendekroos.

Het effect van organische belasting op de verwijderingsefficiënties in ABPs en DBPs is onderzocht en gerapporteerd in **Hoofdstuk 5**. BZV en TSS verwijdering nam toe met de toename in organische belasting in beide systemen. Tijdens de experimentele periode met lage organische belasting zowel als tijdens de periode met hoge organische belasting was de verwijdering van BZV en TSS hoger in het eendekroos-systeem in vergelijking tot het algen-systeem. Vergelijkbare verwijdering van N-totaal werd waargenomen tijdens de lage en de hoge organische belasting in beide systemen. Algehele stikstofverwijdering in ABPs was significant hoger dan in DBPs. N-opname door eendekroos is het eendekroos systeem was 30% en 19% van de totale stikstof belasting tijdens de perioden met lage en hoge organische belasting. Organische belasting had geen effect op de verwijdering van P-totaal in zowel ABPs als DBPs. Hogere fosfor verwijdering werd bereikt in DBPs in vergelijking tot ABPs. In beide perioden was de verwijdering van FC hoger in het algen-system dan in het eendekroos-systeem. Verwijdering van fecale coliformen in ABPs tijdens de periode met lage organische belasting en tijdens de periode met hoge organische belasting was respectievelijk 3.8 en 3.4 log eenheden. In DBPs werd 2.2 en 1.76 log eenheden verwijdering waargenomen tijdens de twee genoemde perioden.

In de literatuur zijn tegengestelde conclusies getrokken omtrent het belang van ammoniak vervluchtiging in vijvers voor afvalwater stabilisatie. In tegenstelling tot eerdere studies, welke alleen influent en effluent-concentraties stikstof gebruikten om ammoniak vervluchtiging te voorspellen, werd in de huidige studie een methode gebruikt waarmee de werkelijke ammoniak flux door dit mechanisme gemeten kon worden (**Hoofdstuk 6**). In deze studie is de ontwikkeling van een eenvoudige methode beschreven voor het direct bepalen van de ammoniak vervluchtiging in vijvers voor afvalwater stabilisatie. De ontwikkelde methode kan ook gebruikt worden voor de kwantificering van de bijdrage van andere zuiveringssystemen aan gas emissies met een mogelijk negatief milieu-effect (bv. CH_4, NO_x, H_2S). Ammoniak vervluchtiging werd bepaald in een continu bedreven proefinstallatie gedurende een periode van 18 maanden. Ammoniak vervluchtiging was hoger in ABPs dan in DBPs. De vervluchtiging correleerde goed met de concentratie vrije ammoniak (NH_3) in de waterfase. Lagere waarden van NH_3 in DBPs vanwege lagere pH waarden resulteerden in lagere ammoniak vervluchtiging in vergelijking tot ABPs. Wanneer de ammoniak concentratie in beide systemen gelijk was, dan was ook de vervluchtiging gelijk. Blijkbaar verminderde de eendekroos bedekking de ammoniak vervluchtiging niet door een fysiek mechanisme. Vervluchtiging was tussen de 7.2 tot 37.4 mg-N $m^{-2}d^{-1}$ en 6.4 tot 31.5 mg-N $m^{-2}d^{-1}$ in respectievelijk ABPs en DBPs. Het belang van ammoniak vervluchtiging in de vijvers van de proefinstallatie was beperkt tot minder dan 1.1% van de totale stikstof belasting van het systeem.

Het verschil in milieuconditities in de vijvers, vanwege de aan- of afwezigheid van de laag eendekroos, veroorzaakte verschillen in de nitrificatie en denitrificatie snelheden (**Hoofdstuk 7**). Hoger DO concentraties in ABPs, in het bijzonder tijdens het warme seizoen, veroorzaakte hogere nitrificatie snelheden in ABPs dan in DBPs. Nitrificatie snelheden waren verder in het systeem niet hoger dan in de eerste vijver ondanks de afname van de concentratie organische stof en de bijbehorende toename in DO. In beide systemen werd denitrificatie (160-560 mg-N $m^{-2}d^{-1}$) vooral waargenomen in het sediment, alwaar anaërobe condities heersten. Nitrificatie en denitrificatie snelheden waren in beide systemen hoger bij hogere temperatuur (21 ^{o}C) dan bij lagere temperatuur (11 ^{o}C). De bijdrage van denitrificatie aan stikstofverwijdering in ABPs en DBPs kwam overeen met 15-25% van de totale stikstof belasting.

Op basis van de stikstof verwijderingssnelheden, door middel van de verschillende mechanismen, bepaald in de voorgaande hoofdstukken werd een stikstof massabalans opgesteld voor zowel de algen- als de eendekroos-vijvers (**Hoofdstuk 8**). Onafhankelijk van de organische belasting en het seizoen was de belangrijkste stikstof flux in ABPs sedimentatie en in mindere mate denitrificatie. In DBPs waren sedimentatie en denitrificatie van gelijk belang, behalve gedurende het warme seizoen en lage organische belasting toen de bijdrage van sedimentatie beperkt was. Stikstof verwijdering door het oogsten van eendekroos was in het bijzonder belangrijk tijdens het warme seizoen en onder lage organische belasting. Ammoniak vervluchtiging in beide systemen was niet meer dan 1.1% van de totale stikstof belasting gedurende de gehele experimentele periode. Incubatie van algen en eendekroos zoals beschreven in hoofdstuk 2 liet hogere ammoniak vervluchtiging zien dan de waarden verkregen in dit hoofdstuk en bepaald in de proefinstallatie. Een beperkte diepte en de hogere pH waarden in de batch incubaties leidden tot een relatief hoge concentratie niet gedissocieerde NH_3 en daarom tot hogere vervluchtiging. Voorspellende modellen voor stikstofverwijdering in ABPs en DBPs zijn voorgesteld in dit proefschrift. De modellen bleken een goede beschrijving te geven van de stikstof stromen en de algehele stikstofbalans onder de aangelegde experimentele condities. Validatie van de modellen met data uit de literatuur resulteerde in slechte resultaten voor ondiepe vijvers, terwijl de modellen beter voorspelden voor diepere vijvers. Verdere uitwerking en validatie van het model is vereist om vijver dimensies en milieufactoren die het functioneren van de vijvers beïnvloeden mee te nemen.

Gevolgen voor ontwerp en bedrijfsvoering van algen en eendekroos-vijvers voor stabilisatie van afvalwater

Twee verschillende doelstellingen voor de behandeling van stikstof houdende afvalwaters in ABPs en DBPs kunnen geformuleerd worden. De eerste doelstelling zou kunnen zijn het maximaliseren van stikstofverwijdering om eutrofiëring van oppervlaktewater tegen te gaan. Een andere doelstelling zou kunnen zijn om de terugwinning van stikstof te maximaliseren voor hergebruik. In ogenschouw nemend de concepten betreffende duurzaamheid en afvalwater management, zoals eerder besproken, kan men concluderen dat technologieën voor afvalwaterzuivering gericht moeten zijn op stikstof terugwinning.
Op basis van deze studie kan geconcludeerd worden dat eendekroos-vijvers het mogelijk maken om stikstof terug te winnen (via opname door eendekroos, sedimentatie en effluent irrigatie) en dit te valoriseren door hergebruik in zowel aquacultuur als agricultuur. Effluent van DBPs veroorzaakt minder problemen voor druppel-irrigatie vanwege de lagere concentratie zwevende stof in vergelijking tot

effluent van ABPs. De geschiktheid van DBPs wordt niet alleen bepaald door de systeem efficiëntie maar ook door de eenvoud van het systeem en de economische waarde van de bijproducten, zoals het eendekroos.

Een afname van stikstofconcentraties van 60 tot 30 mg-N l^{-1} onder de condities van dit onderzoek (21 ^oC) vraagt een hydraulische verblijftijd van 24 en 30 dagen in respectievelijk ABPs en DBPs van 0.9 m diep. Om dezelfde afname in stikstofconcentratie te bereiken moet het oppervlak van de DBPs vergroot worden met 10% t.o.v. het oppervlak van een ABP gedurende de perioden met een hoge organische belasting en met 25% gedurende het koude seizoen. Dit veroorzaakt extra kosten voor land maar het genereert ook inkomsten door eendekroos productie en hergebruik.

Men zou het ontwerp van ABPs zo aan kunnen passen dat sedimentatie gemaximaliseerd wordt en denitrificatie (het belangrijkste stikstof verwijderingsmechanisme) geminimaliseerd wordt, met als doel stikstof terugwinning te maximaliseren. Een betere stikstof verwijdering door sedimentatie vraagt om een combinatie van het stimuleren van stikstof opname door algen-groei en tevens het stimuleren van het afsterven en bezinken van diezelfde algen. Het vergroten van het vijver oppervlak door het verminderen van de diepte zal naar verwachting de algen groei doen toenemen, zoals het geval is in High Rate Algae Ponds, maar dit resulteert ook in een toename van de kosten voor land. Het in een vijversysteem opnemen van een eendekroos-vijver (waar algen bezinken door een gebrek aan zonlicht) die gevoed wordt met algen-vijver effluent zal waarschijnlijk de totale stikstof terugwinning door sedimentatie van afgestorven algen en door opname in het eendekroos doen toenemen. Het verminderen van de diepte van een vijver kan leiden tot een toename van de stikstofverwijdering door ammoniak vervluchtiging, wat vanuit milieu oogpunt onwenselijk is. Daarom is er meer studie nodig naar het optimaliseren van de diepte van een vijver met als doel het stimuleren van sedimentatie en het verminderen van ammoniak vervluchtiging.

Stikstof terugwinning zou geoptimaliseerd kunnen worden door het handhaven van gunstige condities voor de groei van eendekroos. Dit kan bereikt worden door het ontwerpen van vijvers op basis van een juiste organische en stikstof belasting. Het is wordt aangeraden om optimalisatie van groei van eendekroos verder te onderzoeken met in acht neming van het selecteren van de beste specie, optimale dichtheid van de eendekroos bedekking en optimale vijver geometrie.

De op één na belangrijkste mechanismen voor stikstof verwijdering in algen- en eendekroos-vijvers zijn nitrificatie en denitrificatie. Als de waterkolom aëroob gehouden wordt, inclusief de bovenste laag van het sediment (waar de aanwezigheid van nitrificieerders aangetoond is) zou de nitrificatie gemaximaliseerd kunnen worden. Tegelijkertijd zou op deze wijze de denitrificatie geminimaliseerd kunnen worden. Het inbrengen van media in de vijver om meer aanhechtingsoppervlak voor nitrificeerders te bieden is een mogelijk alternatief om nitrificatie te stimuleren.

List of abbreviations

ABPs	algae-based ponds
AF	anaerobic filter
AS	algae systems
ATU	*1-allyl-2-thiourea*
AV	ammonia volatilisation
BOD	biological oxygen demand
C	concentration (mg l^{-1})
CFU	colony forming unit
COD	chemical oxygen demand (mg l^{-1})
CSTR	completely stirred tank reactor
D	dispersion coefficient in (m^2 s^{-1})
DBPs	duckweed-based ponds
DO	dissolved oxygen (mg l^{-1})
DS	duckweed systems
DW	dry weight of duckweed (gm)
FC	faecal coliforms (CFU/100 ml)
HRT	hydraulic retention time (d)
k	nitrogen removal coefficients (d^{-1})
K_b	first-order rate constants of faecal coliforms die-off (d^{-1})
K_l	convection mass transfer coefficient in the liquid phase (d^{-1})
L	length (m)
LiCl	lithium chloride
N	Nitrogen
N_{NH_3}	mass transfer rate of ammonia (mg l^{-1} d^{-1} or mg $m^2 d^{-1}$)
n	number of samples
N	number of stirred tanks in series
NH_3	ammonia
NH_4^+	ammonium
N_{kj}	kjeldahl-nitrogen
NO_2^-	nitrite
NO_3^-	nitrate
NPK	nutrient content (nitrogen, phosphorous and potassium)
OL	organic loading (mg $m^2 d^{-1}$ or kg ha^{-1} d^{-1})
OLR	organic loading rate (mg $m^2 d^{-1}$ or kg ha^{-1} d^{-1})
P	Phosphorus
Q	flow rate (m^3 d^{-1})
RGR	Relative growth rates (d^{-1})
SD	standard deviation
T	temperature (^oC)
t	time (d)
TSS	total suspended solids (mg l^{-1})
U	velocity in (m s^{-1})
UASB	upflow anaerobic sludge blanket
WASCAPAL	water sector capacity building in Palestine
WSP	waste stabilisation pond

Greek

σ^2	variance
α_s	index of short-circuiting
λ_r	removal rate (mg $m^2 d^{-1}$ or kg ha^{-1} d^{-1})
λ_s	surface loading rate (mg $m^2 d^{-1}$ or kg ha^{-1} d^{-1})

ACKNOWLEDGEMENTS

The author are grateful to the Dutch government (SAIL) for financially supporting this research within the scope of the collaboration project “Water Sector Capacity Building in Palestine” between Birzeit University/West Bank and the International Institute for Infrastructural, Hydraulic and Environmental Engineering (IHE)-The Netherlands.

I am grateful to my promotor, Prof. Dr. H. J Gijzen for the valuable discussions in Delft and during visits to Birzeit, comments and ideas along these years of my work. His dedicated attention to my manuscripts was the strongest stimulus to carry this work through.

I am especially indebted to my co-promotor, Dr. Peter Van der Steen who spent many hours discussing and giving advice along these years during the implementation and finalisation of the experimental work at Birzeit University and for his support not only as a supervisor but also as a friend. I want to acknowledge Ir. Siemen Veenstra for his useful discussion during writing the proposal and the initiation of my experimental work.

My thanks to Dr. Bert van Duijl, the “Water Sector Capacity Building in Palestine” project director for his fruitful discussion and for coordinating the project activities and facilitating the financial obstacles.

I gratefully acknowledge Biezeit University and especially the kind cooperation of the Birzeit president Dr. Hana Nasser for his engagement and providing all possible facilities to complete this work. Many thanks go to the employees in the Engineering office in the University for their assistance in constructing the pilot plant (Dr. Bishara Abo Ghanam, Samir Mosa and Mohamed Al-Tokhi). Thanks to the dean of the faculty of engineering Prof. Wael Hashlamoun, my colleagues in the civil engineering department, the director of the Institute for water studies Dr. Munzer Barakat and my colleagues in the master program Dr. Rashed Al-Saed, Dr. Fathi Shakur, Dr. Ziad Mimi Dr. Nedal Mahmoud and Maher Abu-Madi.

Thanks to my sincere colleagues Mostafa Moussa, Mohamed Abu-Thaher, Esi Awuah, Julia Rosa, Rafik Al-Saqaf, Ismail Seyam, Jalal Al-Baz and his family.

It was a valuable experience to work with enthusiastic and dedicated staff and student of Birzeit University. I would like to express my appreciation for the help and support of the laboratory staff members of the MSc. Program at Birzeit (Ms. Abeer Al-Sous and Abdel Haleem Foqaha) for their technical support and for the laboratory assistant (Marwan Ayad) for practical support. Thanks for Saleem Yahya, Arqam Hijjawi, Nuha Ghunem, Mazin Al-Ahmed and Nael Hamdan for their assistance during their MSc. study at Birzeit University. Their findings were very helpful to me.

I would like to thank the Dutch government for financially supporting this research within the collaboration project (WASCAPAL) between Birzeit University, West Bank and UNESCO-IHE, Institute for Water Education, The Netherlands.

Finally, my deeply felt thanks go to my mother, brothers, my lovely wife and my children Basil, Iyad and Tala who without their help I would not have been able to finish my PhD. degree successfully.

CURRICULUM VITAE

Omar Rabah Zimmo was born on November 21st, 1958 in the city of Gaza-Palestine. In 1978, he graduated from Palestine high school in Gaza, after which he enrolled in the Al-Mansoura University in Egypt. He obtained a B.Sc. degree in the civil engineering in 1982 with honors. In 1984 he was awarded a scholarship by the AMIDEAST (USA) to study his MSc. degree in Environmental Engineering in Tufts University, Boston, Massachusetts, USA. His thesis was on “Detection of ground water contamination using ultra violet laser”. After graduation and from 1986 to date, he has been an instructor in the civil engineering department at Birzeit University. In September 1997 he was awarded a scholarship from the Dutch government to study for his PhD in the UNESCO-IHE, Institute for Water Education, The Netherlands. The Netherlands within the cooperation project “Water Sector Capacity Building in Palestine” between Birzeit University and IHE-The Netherlands.

His address in Palestine is, Birzeit University P.O Box 14. West Bank-Palestine.
Telfax: 00972 (02) 298 2120
e-mail: ozimmo@birzeit.edu

Financial support

The research described in this thesis was financially supported by the Dutch government within the collaboration project Water Sector Capacity Building in Palestine (WASCAPAL) between Birzeit University and IHE-UNESCO Institute for Water Education.