NITROGEN TRANSFORMATION AND WASTEWATER TREATMENT EFFICIENCY IN ALGAE-BASED AND DUCKWEED-BASED STABILISATION PONDS

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ABSTRACT

A pilot plant experiment was carried out to determine the differences in nitrogen transformations in two systems for wastewater treatment: algae based stabilisation ponds (ABP) and duckweed (Lemna gibba) based stabilisation ponds (DBP). Each system consisted of a sequence of 4 equal ponds (3m length, 1m width and 0.9 m depth) in series and was fed with a constant flow rate of 0.4 m3/d of partially treated domestic wastewater from Birzeit University. Different nitrogen constituents have been monitored within each treatment system. The results show differences in the nitrogen transformations of the two systems during the 4 months monitoring period (May-August 1999), which started 5 months after the pilot plant start up and lasted for four months. The average total nitrogen (Kj-N+NO2--N+NO3--N) and ammonium (NH4+-N) in pond water were reduced more effectively in ABP (77% and 87% respectively) than in DBP (52% and 60%). Mass balances on total nitrogen indicated that this was because N-loss (probably due to denitrification and ammonia volatilisation) was higher in ABP (32 %) than in DBP (13%). Ammonia volatilisation might be the major part of nitrogen loss in ABP since pH values were greater than the pKa of ammonium (9.3). In, DBP, it's not clear which mechanism (denitrification or ammonia volatilisation) is more important. N-loss in ABP was decreasing in the successive ponds within the system, probably due to reduction in the nitrogen concentration in the pond water. In DBP, N-losses were fairly constant (5-6.5%), except for pond number 1 where N-loss was only 1%. In DBP not nitrogen loss but nitrogen recovery via duckweed harvesting (34%) is the largest contribution to nitrogen removal. Average nitrate concentrations in the final effluent of ABP and DBP were 1.8 and 1.9 mg/L respectively. Higher DO concentrations in ABP (DO was over saturated most of the time) did not favor higher nitrification over DBP (DO range was 1 to 5 mg/L). In ABP, algae seemed to die-off and settle to the bottom of the ponds. This contributed to higher nitrogen accumulation (46% of the influent nitrogen) in comparison with DBP, where only 4% of the influent nitrogen was accumulated as detritus sediment, consisting of dead duckweed biomass. ABP as well as DBP are efficient treatment systems for control of nitrogen in domestic wastewater. Sediment accumulation and removal by volatilisation and/or denitrification in ABP and harvesting of duckweed in DBP are the major nitrogen removal mechanisms for nitrogen control in ABP and DBP, respectively.

KEYWORDS

Algae ponds, duckweed ponds, nitrogen transformations, wastewater treatment

INTRODUCTION

Wastewater treatment, including nitrogen removal, can be achieved in conventional high-tech sewage works at the expenses of high investment and operational cost. In many countries it is

questionable whether sufficient financial resources can be made available for effective treatment technologies. There is therefore a continuous interest for waste stabilisation pond (WSP) systems that are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants. These types of sustainable technologies for wastewater treatment, which are within the economical and technological capabilities of developing countries, need to be developed further. A modified WSP aimed at nutrient recovery is a so called "duckweed pond", covered with a floating mat of small aquatic plants.

Duckweed is reported to have high aerial nitrogen uptake rates, while its biomass is known to be an excellent source of feed for fish or poultry (Skillicorn et al., 1993). Different studies have shown that duckweed systems are capable of treating wastewater (Alaerts et al., 1996; Reddy and DeBusk, 1985). Suspended solids in the effluent is reported to be much lower than for regular WSP, preventing for instance clogging problems by algae material in drip irrigation systems (Pearson et al., 1995). 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). DBP systems are low cost and do not need sophisticated equipment, high energy or qualified labour input. On the contrary, such systems may generate economic return via the commercialisation of biomass for fodder and effluent for irrigation. Most of the available literature on duckweed focuses on the taxonomy, ecology and physiology of the plant. While nitrogen uptake by duckweed has been studied for different types of wastewater, very few studies (Alaerts et al., 1996, Vermaat and Khalid, 1998, Körner and Vermaat, 1998) are available on nitrogen removal via other processes (like nitrification, denitrification and ammonia volatilisation) that take place in the pond system. The exact contribution of various removal mechanisms is not clear.

So far no study has been undertaken to compare the nitrogen transformation processes of conventional ABP and DBP systems under the same operational environmental conditions. Therefore, the aim of this study is to assess, under identical ambient climatic conditions and the same influent wastewater, the performance of the two systems with emphasis on nitrogen transformations and removal processes.

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 $(31^{\circ} 57' 32^{\circ} N, 35^{\circ} 10' 43.8^{\circ} E, 750 above m.s.l)$. The pilot plant was built with reinforced concrete walls to ensure water tightness. It consists of a holding tank followed by two parallel systems: algal based stabilization ponds (ABP) and duckweed based stabilization ponds (DBP). Each system consists of four ponds connected in series (Fig 1). The cross sectional dimensions are 1.3 x 2.2 x 1.9 m (width x length x depth) for the holding tank and 1 x 3 x 0.9 m for the other ponds. Baffles at the outlet of each pond were constructed to avoid short-circuiting and transfer of floating materials to the consecutive ponds.

Pond operation and monitoring

The pilot plant has been operated from December 1998 onwards as a continuous flow system fed with sewage from BZU pumped from an aerated equalization basin with HRT of 1 day. A peristaltic pump to the ABP and DBP pumped the wastewater from the equalization tank at equal rates. Further water transport to subsequent ponds in each train was by gravity. A 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 channeled to 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 coliform (FC) analyses, samples were collected using a sterile 100 ml glass bottles. Dissolved oxygen 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 period were also measured.



Fig 1 Schematic diagram of the pilot plant

Analytical methods

Dissolved oxygen (DO) and pH were measured using a DO175 meter (HACH) and an EC10 pH meter (HACH) respectively. NO2--N and NO₃⁻-N, were analyzed according to the "Advanced water quality laboratory procedures manual" by HACH. The following analytical methods were carried out according to the Standard Methods (APHA, 1992). Chemical oxygen demand (COD) was measured by the dichromate reflux method. Suspended solids (SS) were measured by filtration, and drying of the filter at 105°C. Chlorophyll a concentrations were determined spectrophotometrically at wavelengths of 665and 750 nm after extraction in 90% methanol. The Nessler colorimetric method was used for measuring ammonia. Kjeldahl nitrogen was measured after macro-Kjeldahl digestion followed by distillation and titration. Dry weight of duckweed was measured after drying at 105°C for two days. Nitrogen contents in dried duckweed were measured using a titrimetric method after peroxide digestion according to Novozamsky et al. (1983). Nitrogen content in sediments was measured after drying at 105°C and digestion using the same titrimetric method as described for duckweed. FC counts were determined using the membrane filtration method.

Duckweed and sediment sampling and processing

Sediment samples were collected through pumping by forcing a 2.8 cm glass tube into the sand layer overtopping the pond bottom. Triplicate sediment samples from each pond were collected every two months for analysis of N-content. Duckweed biomass was harvested every fifth day and duckweed density was restored to its initial density of 600-g fresh weight m⁻². Nitrogen removal rates via biomass harvesting were determined by analyzing triplicate samples of stored and dried

harvested duckweed from each pond for total nitrogen content. Duckweed relative growth rates (RGR) were calculated from fresh weights using the equation:

RGR = (ln W2 - ln W1)/t (Hunt, 1978), where W2 and W1 were respectively the final and initial duckweed density during the harvesting cycle of duckweed.

Nitrogen mass balance

The law of conservation of matter was used as the basis for material balance calculations in this study. For both the ABP and DBP, the mass balance equation that was used is the following: $Nin = Nout + Ndw^* + Nloss + Ns$, where,

- Nin = [Kj-N + NO3 + NO2 -] in the influent
- Nout = [Kj-N + NO3 + NO2 -] in the effluent
- Ndw = N removed via duckweed harvesting (This nitrogen component was calculated for DBP only)
- Nloss = N removed via denitrification and ammonia volatilization
- Ns = N-accumulation in the sediment

Results and discussion

Environmental conditions in the ponds

Results obtained from May to August 1999 for the various analytical parameters are presented in Table 1 and 2. In the top 10-15-cm of the water column, higher DO values were observed in ABP (over saturation) than DBP (between 1 and 5 mg/L). Typical diurnal variation in DO concentration in the top 10 cm layer of both system is shown in Fig 2. DO concentrations decreased with the distance from the water surface in all ponds of the two systems and DO concentrations were approximately equal to zero at the lower 30 cm of the water column.



Fig 2. Typical profile of the diurnal variation in the dissolved oxygen concentration of the ABP (a) and DBP (b). 00:00 = 00:00 hours, 20/08/99 and 24 = 00:00 hours 21/08/99

pH values near the water surface were 9.6 - 10.2 and 7.9 - 8.3 in ABP and DBP, respectively. In ABP, pH values increased throughout the line of treatment, whereas in DBP, less variation was observed due to the shading provided by duckweed mat. pH was highest near the surface of water column and slightly decreased with the distance from the water surface. Typical diurnal variation in pH in the top 10 cm layer of both systems is shown in Fig 3. The higher values in pH and DO in ABP were due to algal photosynthetic activities, which was suppressed in DBP as a result of shading by the duckweed mat. Shading caused by duckweed mat also resulted in lowering the water temperature in DBP by at least 1°C in comparison with water temperatures in ABP.



Fig 3. Typical profile of the diurnal variation in the pH of the ABP (a) and DBP (b). 00:00 = 00:00 hours, 20/08/99 and 24 = 00:00 hours 21/08/99

Removal of wastewater constituents

The influent COD ($308 \pm 78 \text{ mg/l}$) to both systems after pre-aeration in the equalization basin was comparatively weak during the study period (equivalent to an average of 140 mg BOD₅/l). In ABP, total COD and TSS values were not reduced through out the pond system due to the growth of micro algae whereas such a growth is prevented in DWP. This finding was consistent with the chlorophyll *a* concentration, which varied between 684 and 2488 µg/l in ABP, and between 44 and 93µg/l in DBP.

Average PO_4^{3-} -P reduction was 78% and 93% in ABP and DBP respectively. Nearly all available PO_4^{3-} -P is exhausted in the forth duckweed pond. The higher reduction of PO_4^{3-} -P in DBP could be attributed to duckweed uptake and subsequent removal by harvesting.

The average faecal coliform (FC) removal in ABP and DBP was 4 and 2 log units respectively. The quality of the effluent from the third duckweed pond (total retention time of 21 days) does not exceed the limit for unrestricted irrigation (WHO 1989). This was achieved already after the second pond in the ABP system (total retention time of 14 days). Less efficient removal of FC in duckweed ponds is due to light effects of the duckweed mat on the pond environment (Van der Steen et al., in press).

Kjeldahl nitrogen (TKN) in the influent was mainly composed of ammonium and only a small portion (5%) of organic nitrogen (Table 2). Throughout the treatment systems, higher values of organic nitrogen were measured in APB (5-12 mg/l) than DBP (2 mg/l) since part of the nitrogen in ABP were incorporated in algal biomass and remained in the water. In DBP, nitrogen removal was less efficient in comparison with ABP. The percentage removal of total nitrogen (TKN+NO2--N+NO3--N) and ammonium (NH4+-N) concentrations in ABP were 77%, 87%, respectively, while in DBP this was 52% and 60%, respectively. The results for ABP are comparable to those of Silva (1982) who obtained overall nitrogen removal of 81% in system of similar depth (1.0 m) and hydraulic retention time (29 days). However, Middlebrooks *et al.*, (1982) reported higher removal value in systems with very long hydraulic retention times of 227 days. The nitrogen removal rate in the duckweed pond system was 1.1 g-N m⁻² d⁻¹. It was higher than values of 0.32 g-N m⁻² d⁻¹

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Table 1: Analyses influent and effluents of the four ponds of each treatment system (ABP and DBP) during the four months monitoring period. All values are in mg/l unless otherwise stated.

Parameter	Inf.	Algae ponds				Duckweed ponds				
		A1	A2	A3	A4	D1	D2	D3	D4	
pH (-)	7.7	9.6	10	10.4	10.2	7.9	8.2	8.4	8.3	
DO	0.1	19	19	19	19	1.7	4.8	6.2	5.1	
T (°C)	26.0	29.0	27.8	27.7	27.6	25.7	26.1	26.3	26.3	
COD (total)	308 ± 78	286 ± 66	284 ± 72	304 ± 29	306 ± 9	197 ± 61	153 ± 37	154 ± 53	141 ± 44	
COD (filtered)	98 ± 10	77 ± 8	76 ± 7	79 ± 17	72 ± 22	82 ± 12	69 ± 12	64 ± 18	64 ± 21	
TSS	253 ± 50	202 ± 28	189 ± 35	150 ± 36	136 ± 31	102 ± 18	88 ± 28	68 ± 20	56 ± 22	
Chlorophyll a (µg/l)	70 ± 37	2488 ± 1250	1169 ± 481	1004 ± 486	684 ± 502	80 ± 44	82 ± 46	93 ± 63	44 ± 35	
PO ₄ ³⁻ -P	4.3 ± 0.4	2.9 ± 0.7	2.5 ± 0.8	1.8 ± 0.8	0.9 ± 0.2	2.8 ± 0.6	1.5 ± 0.7	0.8 ± 0.4	0.3 ± 0.3	
Total-N	65 ± 6	47 ± 8	33 ± 7	21 ± 1	15 ± 2	57 ± 6	48 ± 6	39 ± 3	31 ± 4	
FC (CFU/100 ml)	2.03E+04	4.00E+03	2.53E+02	2.70E+01	4.00E+00	7.37E+03	2.56E+03	5.83E+02	3.83E+02	

Table 2: Nitrogen compounds in influent and effluent of each pond in ABP and DBP during the four months monitoring period. All values are in mg/l unless otherwise stated.

Parameter	Inf.	Algae ponds				Duckweed ponds				n
		A1	A2	A3	A4	D1	D2	D3	D4	_
TKN	64 ± 7	43 ± 8	28 ± 5	19 ± 4	14 ± 5	56 ± 7	44 ± 7	36 ± 6	27 ± 4	14
NH_4^+-N	60 ± 6	30 ± 8	19 ± 6	12 ± 3	8 ± 2	53 ± 6	42 ± 6	33 ± 5	24 ± 4	18
NO_2^N	0.02 ± 0.0	1.8 ± 1.3	2.4 ± 0.7	1.06 ± 0.44	0.39 ± 0.14	0.13 ± 0.04	0.77 ± 0.56	1.29 ± 0.82	1.59 ± 0.67	8
NO ₃ ⁻ -N	0.16 ± 0.16	1.9 ± 1.07	2.7 ± 1.44	1.47 ± 1.01	0.66 ± 0.42	1.08 ± 0.67	2.48 ± 1.36	1.98 ± 0.91	1.95 ± 1.15	18
Organic-N	3 ± 1	12 ± 3	8 ± 3	6 ± 3	5 ± 3	2 ± 1	2 ± 1	2 ± 1	2 ± 2	14

reported by Alaerts et al., (1996) probably due to lower nitrogen concentration of the wastewater used in their experiment. Whereas, 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.

Nitrogen mass balance

Based on the mass balance calculation, the various percentages of different nitrogenous fluxes and removal efficiencies from DBP and ABP were calculated as shown in Fig 4. Also, the percentage of different nitrogenous fluxes at various ponds of the two systems expressed with respect to total influent nitrogen to these ponds are presented in fig 5.







Fig 5. The percentage of different nitrogenous fluxes at various ponds expressed with respect to total influent nitrogen to these ponds: (a) algae based ponds and (b) duckweed based ponds

Nitrogen transformations and removal processes in ABP and DBP

Nitrification/denitrification. The less exposure to inhibitory action of UV light due to duckweed mat in DBP and high suspended solid concentration in ABP may play a role in protecting the nitrifiers. In addition, the long retention time in both systems enabled the slow growing nitrifiers to maintain themselves and therefore nitrification is taking place. Higher nitrite and nitrate concentrations were found in the effluent of ponds 1 and 2 of the ABP in comparison with the corresponding ponds in DBP. This means that net nitrification (Nitrification-denitrification) is higher in ABP than in DBP. The oxygen concentration in the upper 40 cm of the water column during daytime and to lower extent during the night (see fig 2) indicates that oxygen availability

was sufficient for nitrification in both systems. Higher DO in ABP did not favor higher nitrification over DBP. Except for the top layer of the water column in ABP, pH values in both systems were close to the optimal pH values for nitrification reported in literature: 7.3-8.6 (Metcalf and Eddy 1991) and 9 (Azov and Tregubova, 1995). Although, DO and pH were favourable for nitrification in both systems, nitrate was not accumulating along the line of treatment suggesting that denitrification is taking place. This contradicts Reed's statement (1985) that denitrification is only a theoretical possibility for permanent nitrogen loss in ponds. Denitrification is known to occur under anoxic conditions, pH between 7-8 and the presence of enough organic matter. Apparently those conditions are met in the sediment layer in all ponds of the two systems.

Nitrogen loss. Based on the mass balance for the four ponds of each system, the overall nitrogen loss in ABP and DBP through ammonia volatilisation plus nitrification-denitrification were 32% and 13% respectively. Differences in nitrogen losses were calculated in each pond of the two systems. In ABP, except for pond number 1 where N-loss was 11%, nitrogen losses were decreasing in successive ponds (17%, 12% and 7% respectively), most probably due to the reduction of nitrogen content of the water. In duckweed ponds, except for pond number 1 where Nloss was only 1%, N-losses were in the range of 5-6.5% of the influent nitrogen concentration. These values were lower than reported values by Zimmo et al., (in press) in batch experiments with lower hydraulic retention time. In ABP, it is difficult to know which process for nitrogen loss (ammonia volatilisation or denitrification) was more important since favourable conditions for both processes were found in all ponds. In DBP, denitrification was probably responsible for substantial nitrogen loss as very low dissolved oxygen concentration was measured in the lower half meter of the ponds and ammonia volatilisation might not be significant since pH values were lower than the pK_a of ammonium concentration. The possibility that both ammonia volatilisation and denitrification processes could take place in ABP may explain the higher nitrogen loss found in comparison with DBP. The negligible percentage of nitrogen loss in the first duckweed pond (1%) could be attributed to the small amount of denitrification that take place in the pond with nitrification being the bottleneck. By assuming volatilisation in DBP was negligible, the denitrification rates can be calculated as 273 mg-N m⁻²d⁻¹. This value is comparable to the reported values by Vermaat and Hanif (1998); 260 mg-N m⁻²d⁻¹ but rather higher than the reported values by Körner and Vermaat (1998); 10-50 mg-N m⁻²d⁻¹ probably due to the short retention time (3 days) and shallow water depth (3.3 cm) used in their experiments.

Nitrogen accumulation in sediment. Nitrogen accumulation in the sediments of algal ponds was higher than that of duckweed ponds. Based on the mass balance for the two systems, the total contribution of sedimentation to nitrogen removal in ABP and DBP can be calculated as 46% and 4% respectively. In contrast to DBP, the accumulation of nitrogen in the sediment of ABP was the largest single contribution the conversion of nitrogen. The settling solids in the four ABP were 31, 18, 25, and 17 g dry weight m⁻² d⁻¹ and 3.2, 3, 2.7 and 1.3 g dry weight m⁻² d⁻¹ in DBP respectively. The nitrogen content of sediment was in the range of 0.035 and 0.04 g-N g⁻¹ dry weight in ABP and 0.025 and 0.03 g-N g⁻¹ dry weight in DBP. Therefore the percentage removal by sedimentation in the four ABP were respectively 17%, 12%, 23% and 24% of the influent nitrogen in corresponding pond and not more than 1.5% of the influent nitrogen to the correspondent pond in DBP. In duckweed systems, Van der Steen et al. (1998) mentioned higher values of suspended solids sedimentation (6-11 g dry weight m⁻² d⁻¹). Paredez (1999) reported low values (0.08 g dry weight m⁻² d⁻¹) in the first pond of a series of ponds due to absence of solids in the artificial sewage used. The higher nitrogen removal via sedimentation in ABP in comparison with DBP was due to

nitrogen uptake by algal biomass in ABP that eventually settled to the bottom of the ponds and remained in the sediment. This finding is in consistent with the simulation study using actual pond data from WSP in the USA by Ferrara and Avci (1982) in which they found that sedimentation was the main removal pathway. In DBP, duckweed cover prevents sunlight penetration and consequently algae will not developed as in algal ponds. Therefore sedimentation will only be limited to the Settleable suspended solids originally found in wastewater and to the detritus duckweed plant that settle to the bottom of the ponds. The smaller amount of sediment produced in duckweed ponds in comparison with algal ponds will resulted in less frequent desludging and provide an advantage for better sludge management in duckweed pond system.

Nitrogen in duckweed. The uptake of nitrogen by duckweed was the largest single contribution to the conversion of nitrogen in DBP. In the four duckweed ponds about one third of the nitrogen load (35%) may be recovered as duckweed proteins and possibly reused as animal feed. Based on the mass balance for each duckweed pond, the percentages of nitrogen uptake by duckweed were respectively 9%, 10%, 12% and 13% of the influent nitrogen to the corresponding ponds. High duckweed growth was observed in the four duckweed ponds due to the availability of NH4⁺ and PO4³⁻-P. Mean values for the relative growth rates (RGR) of Lemna gibba in the four duckweed ponds were respectively 0.2; 0.19; 0.2 and 0.17 g g⁻¹ FW day⁻¹. The minimum RGR (0.01) was observed during the period between 10 till 24 July 1999. During this period duckweed was infested with aphids, and fronds were turned yellowish, got much smaller in size and lacked the gibbous morphology. Dimethoate insecticide was applied at concentration of 1 ppm and spread three times during the last week of July, and aphids were reduced substantially but were not completely eliminated. The nitrogen uptake rates in the four duckweed ponds were 781, 751, 742 and 669 mg N m⁻² d⁻¹ respectively with an average value 736 mg N m⁻² d⁻¹. It was expected that the RGR and nitrogen uptake rates of duckweed would decrease along the line of treatment due to decrease in micronutrients. However, these means were not significantly different from each other (one way ANOVA, P>0.05). The ammonium concentration along the line of duckweed treatment was varying between 60 mg/l in the influent to the first pond to 8 mg/l in the effluent from the forth pond, suggesting that ammonium concentration in this range did not affect the duckweed growth. Comparable values for nitrogen uptake rate were reported by (Oron, 1994, Van der Steen, 1999). Other authors however reported 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) due to differences in the experimental conditions.

Conclusions

Comparative study of a series of four identical algae based and duckweed based stabilisation ponds with total retention time of 28 days in each system, showed that ABP ponds were more efficient to reduce nitrogen than duckweed ponds. In ABP sedimentation and to less extent loss (denitrification and/or ammonia volatilisation) are the major nitrogen transformation mechanisms responsible for N-removal. In DBP considerable part of the nitrogen (34%) is assimilated in duckweed biomass and subsequently harvested and used as protein source. Smaller amount of sediment in duckweed ponds is produced in comparison with algal ponds, which will contribute to better sludge management in duckweed pond system.

It is not possible based on this research to draw definite conclusions about the importance of ammonia volatilisation or denitrification as an important mechanism for nitrogen loss in both ABP and DBP. Currently the issue is being addressed in our research laboratory.

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