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Comparison between algae-based and duckweed-based wastewater treatment: differences in environmental conditions and nitrogen transformations

O.R. Zimmo*, R. Al Sa'ed* and H. Gijzen**

*Birzeit University, Faculty of Engineering, Department of Civil Engineering, P.O. Box 14, Birzeit, West Bank, Palestine

**International Institute for Infrastructural, Hydraulic, and Environmental Engineering (IHE), P.O. Box 3015, 2601 DA Delft, The Netherlands

Abstract In laboratory-scale batch experiments, duckweed (*Limna gibba*)-based and algae-based wastewater containers have been monitored over 15 days in two experiments with different initial total nitrogen concentrations of 50 (experiment 1) and 100 mg-N/L (experiment 2). Clear differences in environmental conditions were observed. High dissolved oxygen (DO) concentrations were observed in the algae-based, compared to duckweed-based, containers. In the algae-based containers the DO range was between 2.1 to 6.6 mg/L and 1.2 to 4.3 mg/L in experiment 1 and 2, respectively, whereas in the duckweed-based containers DO ranged between 1.1 to 3 mg/l and 0.5 to 2.1 mg/L. Higher pH values were measured in algae-based due to algal photosynthetic activity compared to duckweed-based containers where the duckweed mat prevented sunlight penetration and hence algal development. In algae-based containers, the pH range was 7.9 to 8.6 and 8.1 to 8.4 in experiments 1 and 2, respectively, and 7.3 to 7.5 and 7 to 7.6 in the duckweed-based containers. Depending on initial nitrogen concentrations, duckweed-based containers removed between 42%–62% of total nitrogen and between 56%–95% of Kjeldahl nitrogen from the wastewater, while algae-based containers removed between 45%–48% and 48%–58% of total nitrogen and Kjeldahl nitrogen, respectively. Nitrogen loss, probably due to denitrification and ammonia volatilisation, represents 40% of the total nitrogen content of algae-based and duckweed-based containers. However, in duckweed-based containers only 28% of N-loss was observed in containers with higher initial N-content. This study demonstrates that there were differences in environmental conditions in algae-based and duckweed-based containers, which have caused differences in nitrogen transformation mechanisms.

Keywords Algae; duckweed; environmental conditions; nitrogen removal; nitrogen transformations; wastewater treatment

Introduction

Sustainable technologies for wastewater treatment, within the economical and technological capabilities of developing countries, need to be developed. These technologies should aim to reuse not only water but also the nitrogen content. Both conventional waste stabilisation ponds (WSP) and duckweed-covered ponds offer possibilities for nitrogen reuse, either through effluent irrigation or through animal feed production. Conventional waste stabilisation ponds are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants. Effluent from WSP characterised with high algal concentrations of about 200 mg TSS/L, cause severe clogging problems in advanced (drip) irrigation systems (Pearson *et al.*, 1995).

Duckweed-based ponds (DBP) are stabilisation ponds with a floating mat of small plants on the pond surface. These small plants are generally called duckweed. Different studies have shown that duckweed systems are capable of treating wastewater (Alaerts *et al.*, 1996; Reddy and DeBusk, 1985). The advantage of duckweed systems for nutrient recycling and utilisation is based on the high growth rate of duckweed (Hillman, 1961; Landolt, 1986) and its high protein content of up to 40% of plant dry weight (Rogers *et al.*,

1978; Culley *et al.*, 1981; Mbagwu and Adeniji, 1988). DBP systems might have several advantages over WSP systems: algal blooms are prevented by the presence of a duckweed mat, while nitrogen in the water can be effectively reduced by regular harvesting of the duckweed biomass. Three different mechanisms, apart from duckweed uptake, have been reported in the literature for nitrogen removal in ponds system: ammonia volatilisation, ammonia assimilation into algal biomass and biological nitrification coupled with denitrification. However, it is not clear which process is responsible for most nitrogen loss. As far as we know there are no studies comparing algae and duckweed wastewater treatment systems for nitrogen transformations and removal efficiencies. This study was designed to find out the differences in the environmental conditions in algae and duckweed wastewater treatment containers and to compare the differences between the systems in nitrogen transformation and removal efficiency.

Materials and method

Experimental set-up

Two batch experiments (1 and 2) with different initial total nitrogen concentrations (50 and 100 mg N/L respectively) were conducted to determine the differences in environmental conditions and nitrogen transformations in algae-based and duckweed-based containers. Two batches of wastewater, taken two days apart, from the effluent of the anaerobic pond at the pilot plant at Birzeit University, were used. Nitrogen content of wastewater was adjusted to the desired nitrogen concentration by adding NH_4Cl . Physicochemical characteristics of the wastewater used in Experiments 1 and 2 are shown in Table 1.

Duckweed-based containers were seeded with 25.5 g fresh weight of *L. gibba* which resulted in a density of 400 g fresh weight/m² or 17.5 g dry weight/m². Algae containers were seeded with algae (obtained by centrifugation of samples from the pilot stabilisation ponds at Birzeit University) to a final concentration of 10 mg/L. The increase in initial nitrogen and phosphorus content by the algae was assumed 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 1.0 and 0.5% of the designated initial nitrogen concentration of 50 and 100 mg/L, respectively. Three replicates of plastic containers for each treatment (12-litre volume, 28.5-cm diameter and 30-cm depth) were used as containers for incubation. The experiments were conducted under laboratory conditions (20°C) and the containers were illuminated with metal halide lamps with a photoperiod of 12 hours. The light intensity was 6200 lux. Water loss by evaporation from algae-based and evapotranspiration from duckweed-based containers was compensated for by addition of de-mineralised water (approximately 100 ml/container every 2 days). Each experiment was subsequently monitored for the period of incubation. Duckweed was harvested once at the end of the fifth day and duckweed density was restored to its initial value of 400 g fresh weight/m².

Table 1 Physicochemical characteristic of wastewater used in Experiments 1 and 2

Parameter	Experiment 1	Experiment 2
Kj-N (mg/L)	51	98
NO ₃ -N (mg/L)	0	0
COD (mg/L)	298	333
Total-P (mg/L)	2.23	2.93
pH	7.9	7.9
DO (mg/L)	0.2	0.2
Temperature (°C)	19.5	19.5

Sampling and analysis

Dissolved oxygen (DO), pH and temperature in containers were measured in the afternoon, approximately 8 hours after starting the light period. DO was measured using a DO175 meter (Hach), while temperature and pH values were measured using EC10 pH meter (Hach). Water samples were taken every two days at 9:00 a.m. from mid-depth of each container since analysis revealed that there was no significant difference of nitrogen concentration at different depths of the container. Kjeldahl-nitrogen (N_{kj}), total nitrogen, nitrite (NO_2^- -N), COD and total-P were analysed according to *Standard Methods* (APHA, 1992). Nitrate (NO_3^- -N) was analysed according to *Advanced Water Quality Laboratory Procedures Manual* produced by Hach. Fresh and dry weight (after drying at 70°C for two days) measurements of duckweed were done at the beginning (typical representative samples), during harvesting and at the end of each experiment. Samples of dried duckweed were analysed for nitrogen and phosphorus tissue contents using titrimetric method (APHA, 1992) after peroxide digestion according to Novozamsky *et al.* (1983). Nitrogen content in sediments was measured after collection and drying at 70°C for two days using titrimetric method after digestion as described for duckweed. Removal coefficients for total nitrogen ($Kj-N + NO_3^-$ -N) and total-P were fitted using the exponential equation $y = z e^{(-k \times t)}$ where z is the intercept, k is removal coefficient day^{-1} and t is the time in days. Removal rates of duckweed were calculated based on the surface area of the container.

Nitrogen mass balance

For duckweed-based containers, the following nitrogen mass balance equation was used:

$$N_i = N_f + N_s + N_{dw} + N_{loss} \text{ (mg-N/container)}$$

Where N_i is the initial nitrogen content of wastewater, N_f is the final nitrogen content in the wastewater, N_s is the nitrogen content in the sediment, N_{dw} is the nitrogen content in duckweed and N_{loss} is the nitrogen loss due to denitrification and ammonia volatilisation. A similar mass balance equation was used for algae-based containers, but without the component of N_{dw} . Similar equations were used for the phosphorus mass balance.

Results and discussion

Only trivial differences were observed in temperature in the duckweed and algae-based containers. The average temperature was about 20°C in algae-based and duckweed-based containers in Experiments 1 and 2. Clear differences in dissolved oxygen (DO) and pH values were observed in algae-based and duckweed-based containers (Figures 1 and 2). In algae-based containers the range of DO was between 2.1 to 6.6 mg/L and between 1.2 to 4.3 mg/L in Experiments 1 and 2, respectively, and between 1.1 to 3 mg/l and 0.5 to 2.1 mg/L in duckweed-based containers. The absence of algae in duckweed-based containers due to the shading provided via duckweed mat led to a reduction of DO levels. Oxygen production from suspended algae was absent, whereas the duckweed mat may reduce oxygen diffusion from the air into the water phase. Duckweed might supply some oxygen to the water via transport of oxygen through the root zone (Moorhead and Reddy, 1988), but this contribution is expected to be minor. The high pH values that are usually observed in algae-based cultures due to algal photosynthetic activity did not occur in duckweed-based containers. In the algae-based containers, the pH range was between 7.9 and 8.6 and between 8.1 and 8.4 in Experiments 1 and 2, respectively; and between 7.3 and 7.5 and between 7 and 7.6 in the duckweed containers.

The observed differences in the physicochemical and environmental conditions in the two systems are likely to affect nitrogen transformations and removal processes in the containers. Nitrate concentrations in duckweed-based containers were higher than that in

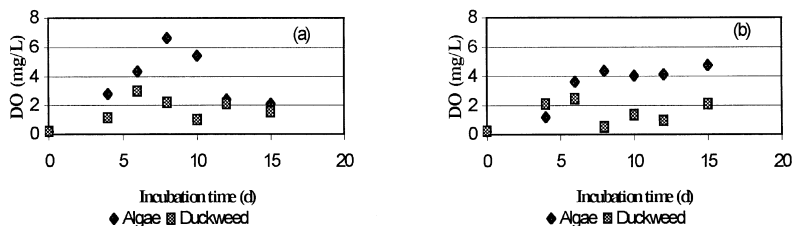


Figure 1 DO concentrations (mean of three replicates) with time of incubation of Experiment 1 (a) and Experiment 2 (b)

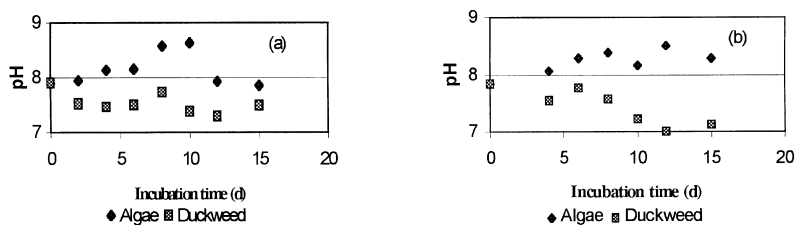


Figure 2 pH values (mean of three replicates) with time of incubation of Experiment 1 (a) and Experiment 2 (b)

algae-based throughout the experimental run although dissolved oxygen concentrations in algae-based were higher. In Experiment 1, NO_3^- -N concentrations after 15 days of incubation in algae-based and duckweed-based containers were 5.3 and 14.8 mg/L, respectively. In Experiment 2 the concentrations were 3.2 and 11.9 mg/L. The shallow depth of algae-based and duckweed-based containers (30 cm) favoured the development of aerobic conditions, which enhanced the nitrification process. Nitrite concentrations were very low and can be neglected. Depending on initial nitrogen concentrations, duckweed-based containers removed 42%–62% of total nitrogen and 56%–95% of Kjeldahl nitrogen from the wastewater, while algae based containers removed 45%–48% and 48%–58% of total nitrogen and Kjeldahl nitrogen respectively. The higher removal of total and Kjeldahl nitrogen in the duckweed-based containers was attributed to nitrogen uptake by duckweed and subsequent removal via duckweed harvesting. In the algae-based containers, the higher final content of Kjeldahl nitrogen in wastewater compared to duckweed-based containers was attributed to the algal biomass present. After 15 days incubation, 40% of the total nitrogen content of the algae-based containers was unaccounted for (loss via denitrification and ammonia volatilisation) for both 50 and 100 mg/L initial nitrogen concentrations. The same percentage of nitrogen was lost in the duckweed-based containers with 50 mg/L initial nitrogen concentration, while only 28% was lost in the container with initial nitrogen concentration of 100 mg/L. The percentage of N present in different nitrogen components after 15 days of incubation from the total N present at the start of the Experiments 1 and 2 are presented in Figures 3 and 4, respectively.

Removal efficiencies of nitrogen in duckweed-based containers were consistent with the values reported for other duckweed systems (Harvey and Fox, 1973; Sutton and Ornes, 1975; Oron *et al.*, 1987; Alaerts *et al.*, 1996). Vermaat and Hanif (1998) and Körner and Vermaat (1998) reported higher nitrogen removal (in 3 to 5 cm containers depth), most probably due to the high duckweed biomass per water volume ratio of the containers used. Removal of nitrogen in algae-based containers in our experiments is difficult to compare with values reported in literature for waste stabilisation ponds which varies from a negligible percentage (Silva *et al.*, 1987) up to 95% (Middlebrooks *et al.*, 1982) depending on system configuration and operational parameters of the ponds.

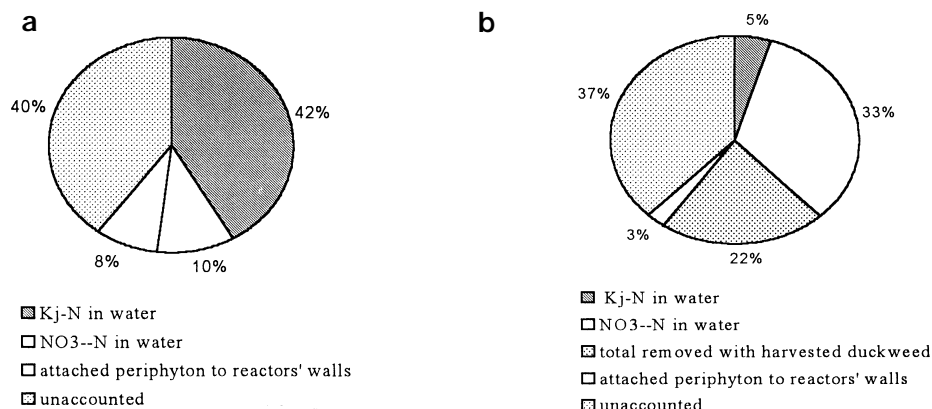


Figure 3 Percentage of N present in different components in algae-based (3a) and duckweed-based containers (3b) obtained after 15 days of incubation from the total N present at the start of Experiment 1

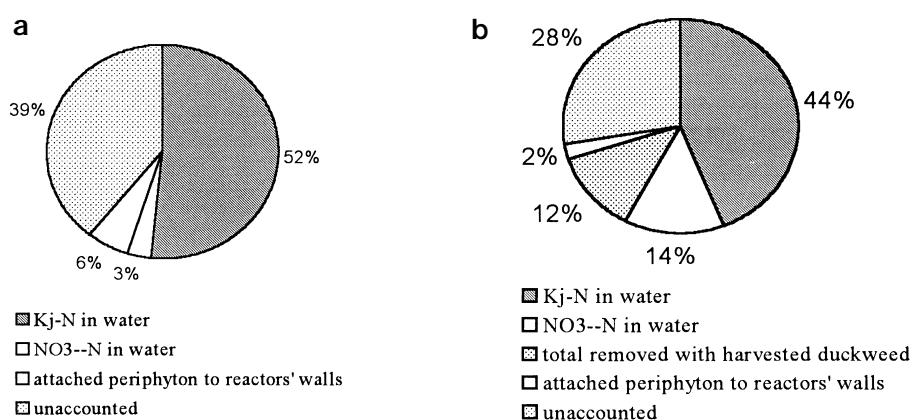


Figure 4 Percentage of N present in different nitrogen components in algae-based (4a) and duckweed-based containers (4b) obtained after 15 days of incubation from the total N present at the start of Experiment 2

In duckweed-based containers, denitrification is probably the major nitrogen loss as very low ammonia volatilisation could be expected in the pH range 7–7.5. Denitrification in duckweed-based containers in our study was higher than the value reported as unaccounted nitrogen loss of 8–10% by Alaerts *et al.* (1996). In the algae-based containers, ammonia volatilisation rather than denitrification could have been the major nitrogen loss since high pH values were measured, while the prevailing aerobic conditions were not favorable for denitrification.

For Experiments 1 and 2 only 7 to 8% of the total phosphorus balance was unaccounted for. The percentage of P in different P components after 15 days of incubation from the total P present at the start of Experiments 1 and 2 is presented in Figures 5 and 6, respectively.

Lower values of total-P (4% and 26% of initial total P present at the start of Experiments 1 and 2, respectively) remained in duckweed-based containers compared to algae-based (74% and 69%). This is basically attributed to the P-uptake by duckweed, which accounted for 80% and 58% of initial total-P in Experiments 1 and 2, respectively. P-uptake rates of duckweed in our experiments (20 mg P/m²/d) were lower than values reported elsewhere in the literature (Reddy and DeBusk, 1985: 87 mg P/m²/d in summer, 18 mg P/m²/d in winter; Zirschky and Reed, 1988: 220 mg P/m²/d). The lower initial total-P in our experiments was probably the main reason for the lower uptake rate.

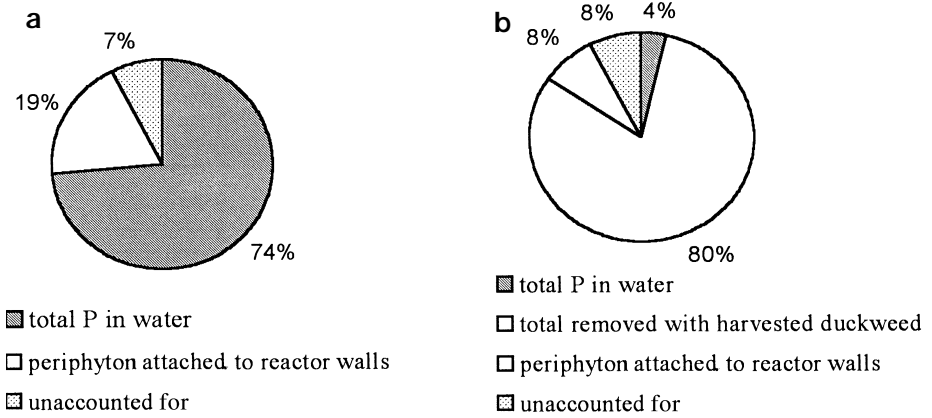


Figure 5 Percentage of P present in different phosphorus components in algae-based (5a) and duckweed-based containers (5b) obtained after 15 days of incubation from total P present at the start of Experiment 1

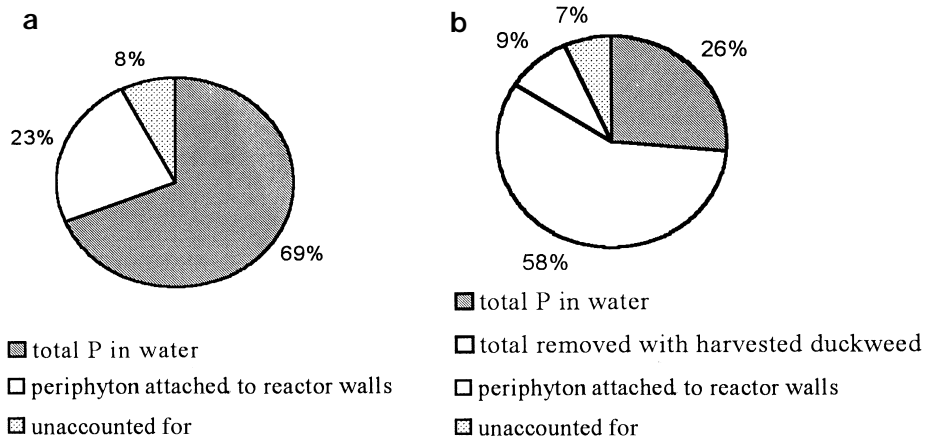


Figure 6 Percentage of P present in different phosphorus components in algae-based (6a) and duckweed-based containers (6b) obtained after 15 days of incubation from the total P present at the start of Experiment 2

Table 2 Final total nitrogen and phosphorus concentrations in Experiments 1 and 2. Values represented as means of three replicates \pm SE

	Container	Total N mg/L	N removal coefficient (d^{-1})	Total P mg/L	P removal coefficient (d^{-1})
Experiment 1	Algae	26.72+0.29	0.05	1.64+0.03	0.02
	Duckweed	16.85+0.43	0.18	0.08+0.003	0.31
Experiment 2	Algae	53.98+0.60	0.05	2.05+0.04	0.03
	Duckweed	55.57+1.07	0.05	0.77+0.03	0.10

The overall nitrogen and phosphorus removal of duckweed-based containers was more efficient in Experiment 1 where the nitrogen concentration was 50 mg/L. At the higher concentration (100 mg/L), overall removal nitrogen removal seems to be similar in the duckweed and algae-based containers. However, better removal of phosphorus was observed in the duckweed-based containers. The total concentrations (Kjeldahl and nitrate after 15 days of incubation) and removal rate coefficients of nitrogen and phosphorus in wastewater at the end of Experiments 1 and 2 are presented in Table 2. Nitrogen and phosphorus

removal coefficients in duckweed-based containers were lower than data reported by Körner and Vermaat (1998) (3–5 cm container depth), most probably due to the high duckweed biomass per water volume ratio of their containers.

Conclusion

The results of this study showed that there was a clear difference in DO and pH between duckweed and algae-based culture systems. These differences caused the changes observed in nitrogen transformations. Nitrification in duckweed-based containers was higher than in algae-based containers, despite the fact that oxygen concentration was found to be lower in duckweed-based containers. Higher percentages of Kjeldahl and total nitrogen removal were observed in duckweed-based containers, at 50 mg-N/L initial nitrogen concentration. Approximately, equal percentages of Kjeldahl and total nitrogen removal were observed at 100 mg-N/L. The higher removal at 50 mg-N/L was attributed to nitrogen uptake by duckweed and the absence of algal biomass in duckweed-based containers. The amount of nitrogen that was converted to duckweed biomass was similar for 50 and 100 mg-N/L initial nitrogen concentrations.

The loss of nitrogen in algae-based containers was approximately 40% of the initial nitrogen concentration used (50 mg-N/L or 100 mg-N/L). The loss of nitrogen in duckweed-based containers was 28% and 37% of the initial nitrogen concentration of 100 mg-N/L and 50 mg-N/L, respectively. This loss was attributed to the combined effect of denitrification and ammonia volatilisation; however, it is not clear which process was of greater importance. Nitrogen loss due to denitrification in duckweed-based containers might be the main nitrogen loss mechanism since pH measurements during the experiments were between 7–7.6; hence, ammonia volatilisation was not likely to occur. On the other hand, ammonia volatilisation in algae-based was most likely to occur, since a smaller surface area was provided for attachment of denitrifiers compared to duckweed-based containers. In addition, higher pH values between 7.9–8.6 were observed.

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