

Performance of duckweed-covered sewage lagoons in Sana'a, Yemen, depending on sewage strength

F. A. Al-Nozaily and G. Alaerts

ABSTRACT

The performance of a duckweed (*Lemna gibba*) sewage lagoon (DSL) was investigated in non-continuous batch system reactors using high strength sewage under natural environmental conditions in Sana'a. Wastewater effluent from the anaerobic ponds of the Sana'a waste stabilization ponds (WSPs) was used with dilution factors (DF) of 0, 2, 3 and 4. The initial COD concentration range applied was 254–600 mg COD l⁻¹ (150–250 mg BOD l⁻¹) and NH₄⁺ of 25–100 mg N l⁻¹, while the duckweed stock density used was 500 g wet weight m⁻². The duration of the experiments was 10 days with a harvesting frequency of 5 days. NH₄⁺ in this very concentrated Sana'a sewage was possibly the most important limiting factor for growth of *L. gibba*. High pH near the end of the reaction time and lower temperatures at night-time probably also contributed to slower growth. Relative growth rate (RGR) decreased from 0.17 ± 0.04 d⁻¹ at an NH₄⁺ concentration of 23–40 mg N l⁻¹ to around 0.00 d⁻¹ at a concentration of 100 mg N l⁻¹. Fresh wastewater helped to grow duckweed, especially at NH₄⁺ < 50 mg N l⁻¹, while after 5 days, algae proliferation and probably the exhaustion of other essential nutrients started to inhibit duckweed growth. COD removal correlated strongly with the applied initial surface loading. At a higher initial COD loading (λ_s) of 869 kg COD ha⁻¹, the removal loading (λ_r) was 710 kg COD ha⁻¹ 10 day⁻¹, while at a lower initial COD loading of 344 kg ha⁻¹, the removal loading was 210 kg COD ha⁻¹ 10 day⁻¹. Total Nitrogen removal ($\lambda_{r,N}$) increased with initial NH₄⁺ concentration and with initial surface loading (λ_N). At initial nitrogen loading (λ_N) of 28 and 164 kgN/ha, the removal loading ($\lambda_{r,N}$) was 25 kgN/ha.10 d and 148 kgN/ha.10 d, respectively. At the same time, the first order COD kinetic removal rate constant increased from 0.10 to 0.16 d⁻¹ at initial COD concentration of 254 and 621 mg/l, respectively. The total nitrogen kinetic removal constant increased from 0.16 day⁻¹ at NH₄⁺ concentration 93 mg N l⁻¹ to 0.26 day⁻¹ at NH₄⁺ 34 mg N l⁻¹. The high DO and pH encountered under outdoor environmental conditions are probably the main cause of the high N removal compared with removal under laboratory conditions. Therefore, total nitrogen removal was taking place through nitrification/denitrification and probably NH₃ stripping.

Key words | COD, duckweed, *Lemna gibba*, NH₄⁺, sewage lagoons, Yemen

F. A. Al-Nozaily (corresponding author)
G. Alaerts
Sana'a University,
Engineering Faculty,
Sana'a,
Yemen
Fax: +967-1-250514
E-mail: susihe@y.net.ye

INTRODUCTION

Macrophyte-based wastewater treatment systems have several potential advantages compared with conventional treatment systems (Brix and Schierup 1989). The use of duckweed (*Lemna gibba*) has been promoted due to its rapid growth rate (Hillman 1961; Landolt 1986), which results in high levels of nutrient removal (Sutton and

Ornes 1975; Alaerts *et al.* 1996). Duckweed has been grown on pre-settled or diluted domestic sewage (Hammouda *et al.* 1995; Alaerts *et al.* 1996), secondary effluent (Sutton and Ornes 1975) and fish culture systems (Porath and Pollock 1982). Duckweed growth depends notably on light (Al-Nozaily and Alaerts in prep), temperature (Wedge and

Burris 1982; Landolt and Kandeler 1987), nutrient concentrations (Al-Nozaily *et al.* 2000b), pH, wind (Clatworthy and Harper 1962), duckweed mat density (Rejmankova 1982), retention time, $\text{NH}_4^+/\text{NH}_3$ concentration and mixing (Al-Nozaily *et al.* 2000a,b; Caicedo *et al.* 2000). Generally, results suggest that duckweed thrives best on diluted sewage ($\text{COD} = 300\text{--}500 \text{ mg l}^{-1}$; Mandi 1994) partly because high strength sewage is associated with toxic concentrations of NH_4^+ , and partly because duckweed is a less efficient oxygen generator than algae (Al-Nozaily *et al.* 2000a).

Duckweed growth is expected to respond differently to different combinations of wastewater and climatological factors. No such research was conducted for Yemen. This is a precursor study to assess the duckweed growth and treatment efficiency of the duckweed sewage lagoon (DSL) on the Sana'a wastewater. There is a shortage of water in Sana'a and this is reflected in the high strength of the wastewater. Effluent from the anaerobic ponds of the Sana'a waste stabilization pond (WSP) is the preferred feed for the DSL system. This is because it is assumed to be amenable to treatment in a DSL after dilution, owing to the presence of N in the preferable form of NH_4^+ in addition to the other essential nutrients for duckweed growth. The main concern when using DSL after anaerobic ponds is that this wastewater still has a high concentration of NH_4^+ ; above 40 mg N l^{-1} could inhibit duckweed growth and nutrient uptake (Al-Nozaily *et al.* 2000b).

The aim of this study was to assess, under the conditions of Sana'a, on batch-wise operated reactors, the lowest dilution factor that still allows vigorous duckweed growth, and the treatability of the wastewater out of the anaerobic ponds at dilution factors (DF) of 0, 2, 3 and 4. This study is part of a broader study on the assessment of duckweed-covered sewage systems for Sana'a, Yemen.

MATERIALS AND METHODS

Experimental design

Four repeated sets of batch experiments were carried out, at DF 0, 2, 3 and 4 (DF=2 means adding one volume

dilution water to one volume sewage), and for each experiment the same effluent sample of the anaerobic ponds was used, apportioned among the reactors after shaking. The conditions of the sets were such that adaptation of duckweed could be followed. Before each experiment, duckweed was harvested from the previous experiment in the set, rinsed using tap water, and healthy clones were transferred directly into the experimental reactors of the next one so that strains could adapt continuously. The experiments were performed with *L. gibba*, the duckweed species that is sturdy and survives best on wastewater (Vermaat and Hanif 1998; Al-Nozaily *et al.* 2000a,b), and that at the same time occurs in ponds in Yemen (own observation) and has wide distribution in the Middle East (Tackholm 1974; Landolt 1986). The seed culture was collected from an uncovered stormwater receiving pond in Yemen at the beginning of the study period, April and May 1997.

The experimental design and the initial parameter values are tabulated in Table 1. $\text{COD}_{\text{total}}$ values of the concentrated wastewater ranged from 600 to 800 mg l^{-1} ($91\text{--}117 \text{ mg N l}^{-1}$); the $\text{BOD}_{\text{total}}$ values are likely to be in the range of $350\text{--}500 \text{ mg l}^{-1}$.

Inhibition of duckweed growth by NH_4^+ was investigated in terms of biomass production and visually in terms of frond colour and size. Triplicate dark plastic buckets of 7-l volume (0.05 m^2 surface area at 20 cm depth) were used as discontinuous batch reactors for each DF. The collected sewage sample was diluted with local tap water, which comes from groundwater. The reactor contents were not renewed during the 10-day experimental period, thus simulating plug flow conditions. Experiment 1 was stocked with comparatively low seed density of $240 \text{ g wet weight m}^{-2}$ ($12 \text{ g wet weight per reactor}$), while for Experiments 2, 3 and 4, a normal stock seed density (Al-Nozaily *et al.* 2000a,b) of $500 \text{ g wet weight m}^{-2}$ ($25 \text{ g wet weight per reactor}$) was applied.

The containers were subjected to the ambient meteorological conditions (Table 2). The growth was monitored every 5 days by netting all the duckweed and harvesting the increment of the 5 day period after which the original stock density was restored. In Experiment 1, since the seed density was lower, the growth was continued until day 10 before harvesting. After netting the

Table 1 | Experimental design and initial conditions

Experiment	<i>L. gibba</i> seed (g wet weight m ⁻²)	Initial characteristics of the concentrated wastewater				
		Temp (°C)	pH	DO (mg l ⁻¹)	TN* (mg l ⁻¹)	COD _{total} (mg l ⁻¹)
1	240	18	7.1–7.3	0.0	91	600
2		20	6.8–7.1		117	808
3	500	18	6.8–7.3		93	600
4		19	7.0–7.5		93	621

*TN=Kj-N+NH₄⁺; sewage did not contain any significant amounts of organic nitrogen, which contributes to Kj-N. It did not contain any NO₂⁻ or NO₃⁻ but NO₃⁻ was added implicitly with the dilution water.

Table 2 | Meteorological data from the meteorological station, Sana'a Airport, Sana'a, during the experimental period (April and May 1997) (Civil Aviation and Meteorology 1997)

Parameter	April 1997		May 1997	
	Mean daily	Min/Max	Mean daily	Min/Max
Av. air temp. (°C)	18.6	15.4/20.9	20.3	17.0/22.5
Min air temp. (°C)	10.5	8.4/12.8	12.7	8.0/15.2
Max air temp. (°C)	27.7	25.0/29.4	28.6	24.2/31.0
Global radiation (μmol m ⁻² s ⁻¹)	1,164	831/1,429	1,142	696/1,440
Sunlight duration (h)	8.0	3.8/11.3	8.1	2.1/11.6

duckweed from each reactor, it was rinsed with tap water to remove attached algae. Excess water was drained by holding the duckweed inside the net until no more water dropped out of the net. The water attached to the duckweed was removed by placing and rolling the duckweed gently between sheets of tissue paper. Then the total netted duckweed biomass was weighed, and the incremental weight of duckweed removed.

Sampling and analysis

Samples were collected from the top centre of each reactor. Analysis and analytical procedure were carried

out according to Standard Methods (1992). COD was analysed by close reflux method, then colorimetry at 600 nm; Kj-N by digestion (macro-Kjeldahl)- distillation, then colorimetry at 425 nm after nesslerization; NH₄⁺ by colorimetry at 425 nm after nesslerization. NO₂⁻ and NO₃⁻ were analysed colorimetrically at 540 nm using Hach, DR 2000. Duckweed seeding and harvesting was carried out manually, using net, removal of attached water on duckweed biomass then weighing (Al-Nozaily *et al.* 2000a). Frond colour and root length were monitored visually.

The physico-chemical parameters of the liquid (pH, DO and temperature) were monitored three times a day (6 a.m., 12 a.m., 6 p.m.) at 5 cm below the duckweed layer.

Air temperature and humidity were also measured three times a day. COD, Kj-N and NH_4^+ were measured three times during the experimental period of 10 days. To avoid algal presence, and with negligible SS in the influent, COD was analysed based on influent (initial) $\text{COD}_{\text{total}}$ and effluent (after 5 and 10 days) COD_{fit} . GF/C (1.2 μm pores) glass fibre filter papers were used. The water loss due to evapotranspiration was compensated daily with tap water. Rainfall, when occurring, was allowed to compensate for all or part of the evapotranspiration, but during high intensity showers the reactors were covered to avoid splashing or loss of duckweed. The duckweed mat was redistributed when needed.

Relative growth rate (RGR) every 5 days was calculated using Equation 1:

$$\text{RGR} = \ln(w_f/w_i)/t \quad (1)$$

where w_i and w_f are the initial and final wet weight, and t the number of days between the two weighings.

Statistical comparisons were made with procedures in the SPSS software package (Norusis 1996). Comparisons among mean values were made by analysis of variance (one-way ANOVA). Significant ANOVAs were followed by mean comparisons using Tukey's honestly significant difference test. Statistical analyses were reported as significant when $P \leq 0.05$.

RESULTS

Results from all experiments were used to study the duckweed growth, temperature, pH and TN removal, while to study COD, DO and NO_3^- changes in the DSL system, only Experiment 4 (as a representative experiment) was used.

Wastewater characteristics and surface loading

COD loading λ_s reflects the situation at the start of experiment (Table 4). TN loading λ_N reflects the situation at the start of all experiments. TN approximately equals Kj-N,

Table 3 | COD concentration and loadings λ_s at different dilutions at the start of Experiment 4

DF	Surface area (m^2)	Initial $\text{COD}_{\text{total}}$ (mg/l)	λ_s ($\text{Kg COD}_{\text{total}} \text{ha}^{-1}$)
0	0.05	621	869
2		369	517
3		246	344
4		254	356

because NO_2^- and NO_3^- were not detected in the influent but were provided with dilution water, and organic nitrogen was negligible. During the experiment, $\text{TN} = \text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$. NH_4^+ will always mean total ammonium ($\text{NH}_4^+ + \text{NH}_3$) (Table 4).

As the experiments were conducted outdoors, the light intensity during the daytime was much higher than the saturation level. Evapotranspiration was 300–500 ml day^{-1} , which amounts to 6–10 mm day^{-1} in the reactors. The fluctuation of wastewater temperature was affected by ambient air temperature. During all experiments, water temperature was in the range 12–22°C at 6 a.m., 26–32°C at 12 a.m. and 15–22°C at 6 p.m. No diurnal fluctuation of pH was found but pH tended to increase gradually throughout the experiments probably because of metabolism of fatty acids and algal activity. pH of the wastewater at the start of all experiments was around 7. pH increased with time and with higher dilutions. At DF = 0, 2 and 3, pH increased gradually to reach 9 ± 0.3 at the end of all experiments. At DF = 4, pH increased gradually and reached 10.7 ± 0.2 at the end of the experiments.

During Experiment 4, DO increased with time and with increasing DF. DO increased from 0.2 to 5.9 $\text{mg O}_2 \text{l}^{-1}$ at DF = 0 at the start and the end of the experiment, respectively, from 0.2 to 5.1 $\text{mg O}_2 \text{l}^{-1}$ at DF = 2, from 1.2 to 5.2 $\text{mg O}_2 \text{l}^{-1}$ at DF = 3, and from 2.2 to 5.2 at DF = 4. DO was not characterized by pronounced diurnal fluctuation, typical for algae-dominated ponds. At the same time, NO_3^- at DF = 0 increased during the first 3 days from 0 to 8 mg N l^{-1} and then decreased to 0.5 mg N l^{-1} at day 5

Table 4 | Kj-N concentration and loadings λ_N at the beginning of all experiments

DF	Surface area (m ²)	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
		Kj-N* (mg N l ⁻¹)	λ_N (kg N ha ⁻¹)	Kj-N* (mg N l ⁻¹)	λ_N (kg N ha ⁻¹)	Kj-N* (mg N l ⁻¹)	λ_N (kg N ha ⁻¹)	Kj-N* (mg N l ⁻¹)	λ_N (kg N ha ⁻¹)
0	0.05	91	127	117	164	93	130	93	130
2		51	71	83	116	60	84	61	85
3		33	46	55	77	40	56	43	60
4		23	32	25	35	20	28	34	48

*Kj-N=NH₄⁺; sewage did not contain any significant amounts of organic nitrogen, which contributes to Kj-N. It did not contain any NO₂⁻ or NO₃⁻ but NO₃⁻ was added implicitly with the dilution water.

and further increased to 5 mg N l⁻¹ at the end of the experiment. At DF=2 and 3, NO₃⁻ concentration was in the range 0.3–5 mg N l⁻¹ during the first period of 5 days, it then decreased to 0.5 mg N l⁻¹ during the last period of 5 days. At the same time, DO was also fluctuating.

Relative growth rate (RGR)

The net growth rate during the first 5-day growth period increased with decreasing NH₄⁺ concentration (Figure 1). In each experiment, RGR was significantly different

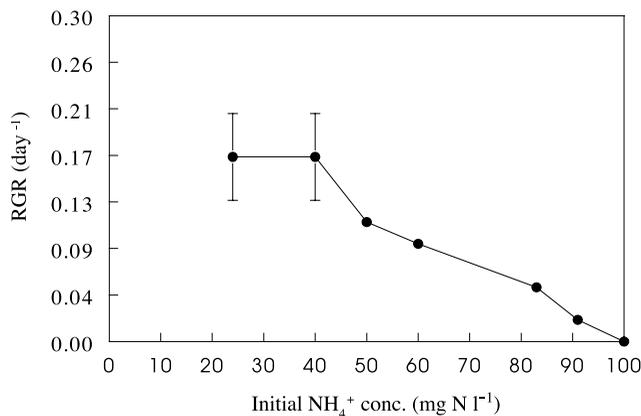


Figure 1 | RGR as a function of initial NH₄⁺ concentration. Average of all reactors, over the first growth period of 5 days. pH ranged from 7–7.5 at day 0 to 9–11 at day 10.

($P \leq 0.05$) with different dilutions except for DF=3 and 4. During the second 5-day growth period, the RGR lowered significantly to very low or even negative values. The growth dramatically decreased to 0.06–0.09 day⁻¹ at 5–24 mg N-NH₄⁺ l⁻¹ and went down to 0.03–0.05 day⁻¹ at 23–30 mg N l⁻¹.

Visual observations

Visual observation corresponds with ammonia concentrations in the wastewater. In all experiments, at DF=0 and with a total ammonium (NH₄⁺ + NH₃) concentration range of 91–117 mg N l⁻¹, *L. gibba* lost its roots within two days, lost its frond pigment, became one-frond clones, and finally died within three to five days. At DF=2, with total ammonium concentrations of 50–83 mg N l⁻¹, fronds were bright and green; roots were short (<1 cm). At DF=3, with total ammonium concentrations of 33–55 mg N l⁻¹, fronds became larger than the original size of the seed, roots were long (2–3 cm) and colour was dark, healthy green. At DF=4 with the lowest NH₄⁺ concentration of 23–34 mg N l⁻¹, during the first five days frond colour was healthy green. However, near the end of the experiment, the fronds tended to turn pale green reflecting the gradual exhaustion of essential growth nutrients. In all experiments at DF=0 and 2, the algal bloom was more pronounced than at higher DF. Algal bloom was observed within two to three days. Algae first started

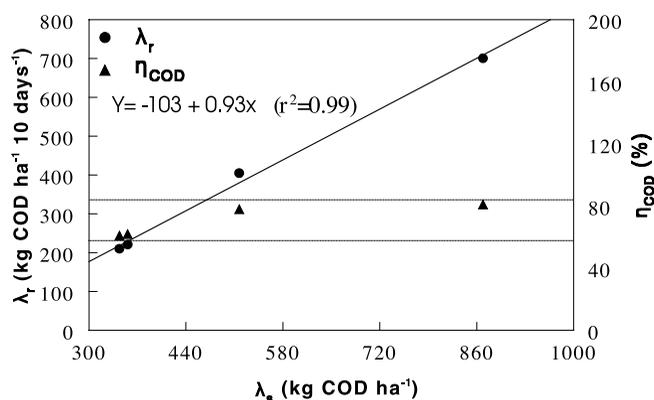


Figure 2 | COD removal λ_r and η_{COD} as a function of λ_s at different dilutions (DF=0, 2, 3 and 4) in Experiment 4. Horizontal lines indicate the range of % removal.

attaching to the duckweed fronds and roots and then spread out to the water column. Once the algae proliferated, it was not possible to grow duckweed even when ammonium concentration reached lower levels after a period of time, indicating the exhaustion of essential nutrients. At DF = 3 and 4, less algae attached to the fronds and roots.

COD removal

COD removal rate in surface organic loading rate terms λ_r ($\text{kg COD ha}^{-1} 10 \text{ days}^{-1}$) increased with initial COD concentration and loading rate applied λ_s (kg COD ha^{-1}) (Figure 2). At higher λ_s of $869 \text{ kg COD ha}^{-1}$, the λ_r was $710 \text{ kg COD ha}^{-1} 10 \text{ days}^{-1}$, while at lower λ_s of 344 kg ha^{-1} , the λ_r was $210 \text{ kg ha}^{-1} 10 \text{ days}$. The removal efficiency η_{COD} also decreased with lower initial COD and λ_s from 81 to 61%. The first order kinetic removal constant also decreased from 0.16 to 0.10 day^{-1} (Figure 3).

TN removal

TN load removal rate $\lambda_{r,N}$ ($\text{kg N ha}^{-1} 10 \text{ days}^{-1}$) increased with initial NH_4^+ concentration and with initial N load λ_N (kg N ha^{-1}) although percentage removal rate decreased (statistically non-significant) (Figure 4). TN removal rate as a function of experiment duration (days)

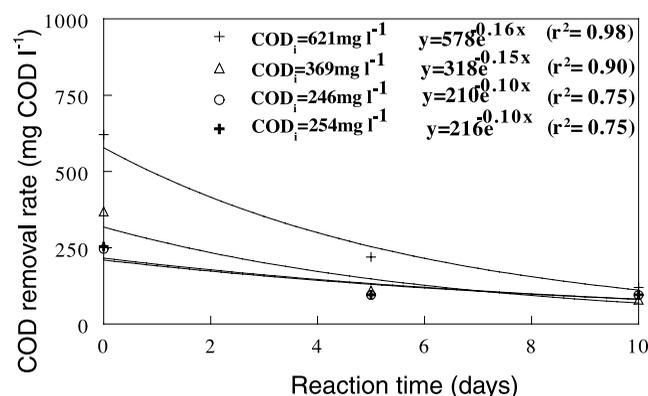


Figure 3 | COD removal rate as a function of experiment duration at different dilutions (DF=0, 2, 3 and 4) in Experiment 4.

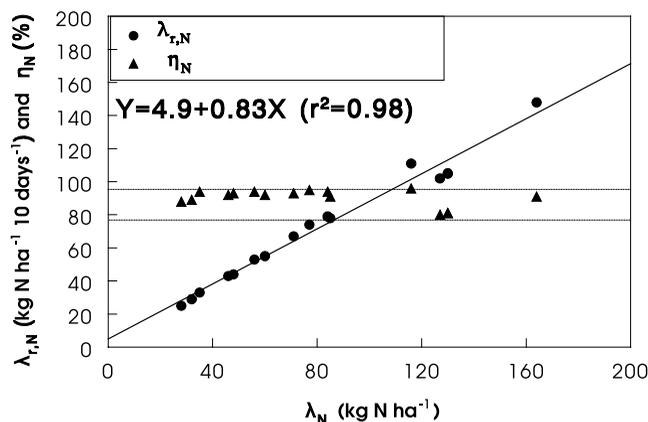


Figure 4 | N removal $\lambda_{r,N}$ and η_N as a function of λ_N in all experiments. Horizontal lines indicate the range of % removal.

followed first-order kinetics. The reaction constant increased proportionally with the increase in DF. In Experiment 4, a representative of the conducted experiments, it increased from 0.16 to 0.26 day^{-1} at DF=0 and 4, respectively (Figure 5).

DISCUSSION

The water temperature ranged from 12 to 22°C in the early morning and late at night. This temperature was lower than the optimum for *L. gibba* of 26°C (Hillman 1961),

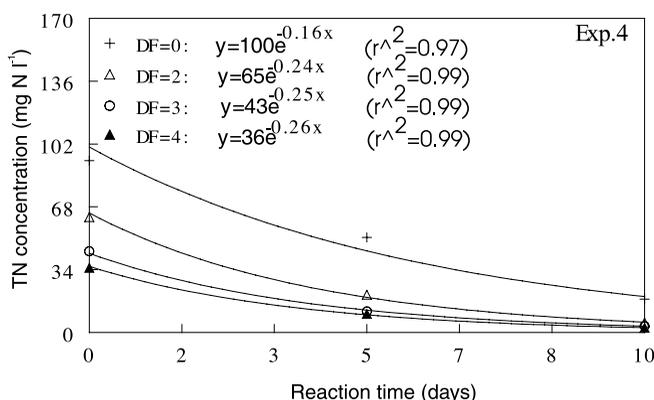


Figure 5 | TN removal rate as a function of experiment duration at different dilutions (DF=0, 2, 3 and 4) in Experiment 4.

which might have some influence on growth. pH at the beginning of the experiment was 7.3–7.5, increasing to around 10 at the end of the experiments. Although algae presence was more pronounced at lower dilution (DF = 0 and 2), the difference between initial and final pH was larger at higher dilution (DF = 3 and 4), which was probably due to a decrease in alkalinity (as a buffering capacity) and metabolization of fatty acids. In contrast, pH in laboratory experiments (Al-Nozaily *et al.* 2000a) did not experience values above 8. Vroon and Weller (1995) and Alaerts *et al.* (1996) also reported a pH range of 7–8 as well as the absence of a diurnal profile or stratification, during the experiments or full-scale DSL. The presence of algae in our experiments pertains to the strong sunshine that caused an increase in light intensity and temperature during the daytime. Optimum pH for duckweed was reported as 4.5–7.5 (Hillman 1961). So, the high pH values near the end of our experiments are considered to inhibit growth.

The strong correlation between deteriorating RGR and the (initial) NH_4^+ concentration strongly suggests that NH_4^+ is the main growth inhibitor. Low stock density in Experiment 1 encouraged comparatively higher RGR, a phenomenon which was also reported by Rejmankova (1982). The safe concentration range for NH_4^+ could be considered to be ≤ 40 –45 mg N l^{-1} . This is consistent with the findings in the lab experiments on wastewater (Al-Nozaily *et al.* 2000b); at initial NH_4^+

concentration of 25, 43 and 96 mg N l^{-1} , the RGR was 0.19, 0.13 and 0.05, respectively, which suggests that $>40 \text{ mg l}^{-1}$ exerted a toxic effect. Das (1998) reported that RGR decreased with an increase of pH and total ammonium. He attributed the toxicity to the ratio of $\text{NH}_3/\text{NH}_4^+$ that increases with an increase of pH. At a controlled pH of 8.3–9, RGR decreased from 0.22 to 0.00 day^{-1} at 10 and 50 mg N l^{-1} . The inhibition in these experiments was thought to be due to the NH_4^+ concentration as well as pH. Growth rate under full daylight of 800–1,100 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ as in Mirzapur, Bangladesh, on domestic wastewater in field conditions resulted in a RGR of 0.23–0.35 day^{-1} for *Spirodela* at NH_4^+ concentration of 8 mg N l^{-1} .

Duckweed stimulated its growth at comparatively high concentration of ammonium but with very slow growth while at lower concentrations the growth was higher. Root development increased with a decrease in NH_4^+ in the solution indicating that roots elongate with the decrease in toxicity of NH_4^+ . However, when the roots become elongated and pale green, this indicates the approach of exhaustion concentrations. This corresponds with the findings of Landolt (1986) for the case of root elongation, and with Landolt and Kandeler (1987) and Das (1998) who reported decreasing root length with increasing total ammonium concentration. At the same time the decrease in frond size at low DF was associated with high total ammonium concentration, which suggests the inhibition of plant metabolic activities due to accumulation of NH_4^+ inside the cells. Das (1998) reported green fronds at 50 $\text{mg NH}_4^+ \text{ l}^{-1}$ with root length of 1.5 cm while at values $>50 \text{ mg NH}_4^+ \text{ l}^{-1}$ fronds started to turn yellowish green with roots $<1 \text{ cm}$. Das (1998) confirmed these results, reporting the presence of algae in all reactors with total ammonium concentrations of 50 mg N l^{-1} and above, which corresponds with conditions that are less favourable for rapid development and maintenance of dense duckweed cover. Once algae has competed and proliferated in the wastewater, it was not possible to grow duckweed even with low NH_4^+ concentrations. During the second 5 days of the growth period using the same wastewater, RGR decreased dramatically. This could also suggest that other essential nutrients are exhausted in the wastewater.

Bacteria present in the duckweed system probably affected COD removal. The kinetic removal constant of $0.10\text{--}0.16\text{ day}^{-1}$ at initial COD concentration of $254\text{--}621\text{ mg l}^{-1}$ in these experiments was found to be higher than that in the laboratory experiments ($0.04\text{--}0.06\text{ day}^{-1}$) on sewage at initial $\text{COD}_{\text{total}}$ of $200\text{--}500\text{ mg l}^{-1}$ ($113\text{--}294\text{ mg COD}_{\text{filt}}\text{ l}^{-1}$) (Al-Nozaily *et al.* 2000a). DO in this study was still low but perhaps higher than in indoor experiments on sewage, which in addition dealt with low soluble COD values. DO addition to the bacteria is better, resulting in more COD removal.

Given that all reactors had the same surface area and volume but different initial COD concentration, this resulted in different initial organic loading. This encouraged increasing the percentage COD removal (within a 10-day period) with loading, from 62% (at initial COD of 254 mg l^{-1}) to 81% (at initial COD of 621 mg l^{-1}). COD load removal λ_r ($\text{kg COD ha}^{-1}\text{ 10 days}^{-1}$) increased linearly with initial COD concentration and with initial COD load λ_S (kg COD ha^{-1}). This corresponds to findings on pre-settled sewage with different surface loadings and with mixing (Al-Nozaily *et al.* 2000a), and with outdoor experiments with *L. gibba* on a supernatant wastewater with initial COD of $250\text{--}400\text{ mg l}^{-1}$ and a removal performance of 63.4% after 10 days (Oron *et al.* 1987). Mandi (1994) also found a comparable value of 72.1% COD removal in 7 days using diluted (at $\text{DF} = 4$) domestic wastewater at initial COD of 444 mg l^{-1} . However, this percentage removal is still low in comparison with the performance of conventional algae-based sewage lagoons, and in a 20-day full-scale DSL with 89–90% removal which operated at low initial pre-settled COD of $250\text{--}300\text{ mg l}^{-1}$ in Bangladesh (Alaerts *et al.* 1996). In the latter case it is possible that physical settling or organic settleable matter marginally influenced the results.

TN removal rate $\lambda_{r,N}$ ($\text{kg N ha}^{-1}\text{ 10 days}^{-1}$) increased linearly with initial NH_4^+ concentration and with initial N load λ_N (kg N ha^{-1}), although percentage removal rate decreased (statistically non-significant). However, the removal performance observed in this study was higher than that in the lab experiments in 20 days (Al-Nozaily *et al.* 2000b). TN removal rate as a function of experiment duration followed first-order kinetics and was also higher ($0.16\text{--}0.26\text{ day}^{-1}$ at initial TN of 93 and 34 mg N l^{-1})

compared with 0.04 day^{-1} at an initial concentration of 25 mg N l^{-1} in the lab experiments. Therefore, it can be deduced that high COD does not inhibit *L. gibba* growth but COD removal will be constrained by the limited oxygen generation capacity of the system.

In addition to duckweed uptake, other removal mechanisms such as nitrification/denitrification might have taken place mainly at lower dilutions of wastewater owing to oxidation of NH_4^+ to nitrate, which might then ultimately be denitrified because of the presence of organic matter and the preference of duckweed to take up NH_4^+ over NO_3^- (Melzer 1980, cited in Landolt 1986; Ullrich *et al.* 1984). DO and NO_3^- fluctuation may have encouraged nitrification/denitrification activity in the reactors at different rates. At $\text{DF} = 0$, denitrification was encouraged more compared with the diluted liquids; this was probably due to the presence of organic matter and anaerobicity as the proper conditions for bacteria that undertake the process. Higher pH, on the other hand, would shift the chemical equilibrium towards NH_3 , and would facilitate ammonia stripping. Algal uptake of nutrients can help to immobilize nitrogen inside the algae, and washout during harvest also contributes to removal, although these mechanisms are not likely to be of major importance (Al-Nozaily *et al.* 2000b).

CONCLUSIONS

Ammonium concentration in the strong wastewater of Sana'a is likely to be the most important limiting factor for the growth of duckweed. High pH near the end of the reaction time and lower temperatures during the night-time probably also contributed to slower growth. RGR decreased from $0.21\text{--}0.13\text{ day}^{-1}$ at NH_4^+ concentrations of $23\text{--}40\text{ mg N l}^{-1}$ to around 0.00 day^{-1} at an NH_4^+ concentration of 100 mg N l^{-1} . RGR of the duckweed decreased with the application of the same wastewater during the last 5 days of the experiment, which could be attributed to the exhaustion of other essential nutrients.

The COD kinetic removal constant increased with initial COD concentration from 0.10 to 0.16 day^{-1} at initial COD concentrations of 254 and 621 mg l^{-1} ,

respectively. λ_r (kg COD ha⁻¹ 10 days⁻¹) increased with initial COD concentration and applied loading λ_s (kg COD ha⁻¹). At λ_s of 869 and 344 kg COD ha⁻¹, λ_r was 710 and 210 kg COD ha⁻¹ 10 days⁻¹, respectively. The best fitting linear equation is COD removal = 0.93 (COD_{total} initial) – 103 with $r^2 = 0.99$.

$\lambda_{r,N}$ (kg N ha⁻¹ 10 days⁻¹) increased with initial NH₄⁺ concentration and with initial λ_N (kg N ha⁻¹). At λ_N of 28 and 164 kg N ha⁻¹, $\lambda_{r,N}$ was 25 kg N ha⁻¹ 10 days⁻¹ (88%) and 148 kg N ha⁻¹ 10 days⁻¹ (91%), respectively. The best fitting linear equation is TN removal = 0.83 (TN initial) + 4.9 with $r^2 = 0.98$. The TN kinetic removal constant increased from 0.16 day⁻¹ at low DF (NH₄⁺ = 93 mg N l⁻¹) to 0.26 day⁻¹ at high DF (NH₄⁺ = 34 mg N l⁻¹).

High DO and pH, which encouraged nitrification/denitrification and probably NH₃ stripping, were the main causes of higher removal rates.

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