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Short communication

The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*

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Abstract

Laboratory scale batch experiments were performed under controlled conditions at different total ammonia concentrations (10–300 mg N l^{-1}) and controlled pH values of 6.8–8.7 using settled domestic wastewater to measure the effect of the ionised (NH_4^+ or ammonium) and un-ionised form (NH_3) on the growth of the duckweed *Lemna gibba*. Relative growth rates (RGR) varied between 0 and 0.3 per day. The toxicity of total ammonia to duckweed was a result of the effect of both, ionised and un-ionised, forms at low NH_3 concentrations (<1 mg N l^{-1}). At higher NH_3 concentrations, the toxic effect of the ionised form could be disregarded. Relative growth rates of *L. gibba* decreased linearly with increasing NH_3 concentrations up to a maximum level (8 mg N l^{-1}), above which duckweed died. These data indicate that *L. gibba* can be used to treat wastewater containing high total ammonia concentrations as long as certain pH levels are not exceeded. Extrapolated relative growth rates resulting from different combinations of pH and total ammonia are given for the examined ranges. Up to a pH of 7.8, a substantial production of 55 kg DW ha^{-1} per day was achieved. Wastewater treatment using *L. gibba* becomes impossible at pH levels above approximately 9.8, depending on the temperature. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Sustainable wastewater treatment; Duckweed; Ammonia toxicity

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1. Introduction

Duckweed-covered systems are one option for sustainable wastewater treatment because of various advantages (Reddy and Smith, 1987; Brix and Schierup, 1989), for example, high nutrient removal efficiencies due to very high growth rates (Hillman, 1961; Landolt, 1986; Vermaat and Hanif, 1998) and the high nutrient content of duckweed (Culley et al., 1981; Lüönd, 1982). Ammonia is one of the major constituents of domestic wastewater and concentrations commonly range from 10 to 50 mg l^{-1} N, but might be as high as 200 mg l^{-1} N in industrial wastewater (Konig et al., 1987) or in domestic wastewater in arid and semi-arid countries (Veenstra et al., 1995). Despite being the preferred nitrogen source by duckweed plants (Porath and Pollock, 1982), it may become one of the parameters inhibiting the growth of duckweed in wastewater (Bitcover and Sieling, 1951; Lüönd, 1982).

Total ammonia in aqueous solution consists of two principal forms, the ammonium ion (NH_4^+) and un-ionised ammonia (NH_3), with relative concentrations being pH- and temperature-dependent. The un-ionised form is most toxic due the fact that it is uncharged and lipid soluble and thus traverses biological membranes more readily than the charged and hydrated NH_4^+ ions (Wuhrmann and Woker, 1948; Downing and Merkens, 1955). A number of studies, therefore, attribute the toxicity of total ammonia to the effect of NH_3 only (Wang, 1991; Clement and Merlin, 1995). In other studies, both forms are reported to become toxic at higher concentrations (Litav and Lehrer, 1978; Monselise and Kost, 1993).

Several studies have been made on the toxicity of ammonia for duckweed (Oron et al., 1985; Wang, 1991; Monselise and Kost, 1993; Clement and Merlin, 1995; Caicedo et al., 2000), but pH was often not controlled which precludes any distinction of the effects of NH_3 and NH_4^+. To date, no final conclusions can be drawn from the present literature regarding the toxicity of either NH_3 or NH_4^+ to duckweed.

In the present study, we used carefully controlled pH ranges to distinguish between the effect of the un-ionised NH_3 and the NH_4^+ ion. The objective was to find the relationship between total ammonia, pH and relative growth rate of the duckweed species *L. gibba* to assess its possible use for the treatment of wastewater with high total N concentrations.

2. Material and methods

Four sets of laboratory scale experiments were carried out using *L. gibba*, a duckweed species that was known to perform well on domestic wastewater (Vermaat and Hanif, 1998; Körner et al., 1998). Duckweed was taken from a stock culture (originally collected in Gouda, The Netherlands) acclimated to domestic wastewater for several months. Each experiment was started with 20 fronds grown in batch reactors (6 cm deep, 64 cm^2 surface area) containing 250 ml settled domestic wastewater from the wastewater treatment plant Hoek van Holland. The wastewater used during the period of study contained 355–465 mg l^{-1} COD, 135–205 mg l^{-1} BOD, 29–52 mg l^{-1} Kj-N, 13–31 mg l^{-1} NH_4^+-N, 0.6–1.7 mg l^{-1} PO_4^{3-}-P and 1.6–2.4 mg l^{-1} total phosphorus (before settling). NH_4Cl was added or the wastewater was slightly diluted (only for the 10 mg l^{-1} treatments) to arrive at desired total ammonia concentrations (NH_4^+-N + NH_3-N) at the beginning of the experiments. The growth medium was changed on the fourth, seventh, and tenth day to prevent growth of algae and compensate for ammonia losses. Duckweed plants were washed and reactors were replaced during each media change to reduce attached algae. The relative concentrations of NH_4^+ and NH_3 are pH dependent, as described by the following equilibrium equation (Erickson, 1985):

$$K_a = \frac{[NH_3][H^+]}{[NH_4^+]} \tag{1}$$

The relative concentrations of the two forms are also temperature dependent (Emerson et al., 1975):

$$pK_a = \frac{0.09108 + 2729.92}{(273.2 + T)} \tag{2}$$

Using pH and temperature of the solution, the un-ionised ammonia fractions can be calculated from the following equation (Clement and Merlin, 1995):

$$\text{Un-ionised } NH_3\ (\%) = \frac{100}{(1 + 10^{(pK_a - pH)})} \tag{3}$$

NH_3-N-concentrations were calculated from the total ammonia concentrations using Eqs. (2) and (3). Total ammonia was determined according to standard methods (APHA, 1992) using a Perstorp Tecator Aquatec auto-analyser. Corrections of pH were done two to three times a day using NaOH or HCl. Therefore, levels of pH varied ±0.4 units at most within each treatment.

Water losses due to evaporation were compensated by adding de-mineralised water once a day. Experiments lasted for 11–12 days. Replicates (three per treatment) were placed randomly under metal halide lamps (Phillips HPIT) at an irradiance of 150–170 μmol photons $m^{-2}\,s^{-1}$ (16 h light, 8 h dark), with a temperature of 22–25°C.

The relative growth rate (RGR) was determined from the dry weight (DW) of *L. gibba* at the beginning of the experiments (DW_0) and at the end (DW_1) using the equation: $RGR = (\ln DW_1 - \ln DW_0)/t$ (Hunt, 1978). DW was determined after drying the plants at 70°C until constant weight.

Two ways of arriving at different combinations of NH_4^+ and NH_3 were used: (1) we varied the total ammonia concentrations at constant pH values in experiments one, two and three, and (2) we adjusted the pH at constant total ammonia concentrations in experiment four. Values of pH during experiments one, two and three were 6.8, 8 and 8.7, respectively, to cover a typical pH range of domestic wastewater. This resulted in un-ionised ammonia fractions of 0.3, 4.7 and 19.8%, respectively (at 23°C), and total ammonia concentrations were 10, 50, 100, 150, 200, 250, 300 mg l^{-1} in experiment one, 10, 50, 80, 120, 200, 250 g l^{-1} in experiment 2 and 10, 30, 50, 80, 100, 150 mg l^{-1} NH_4^+-N+NH_3-N in experiment 3. During experiment 4, total ammonia concentrations were kept constant at 50, 100 and 150 mg l^{-1} and pH values were adjusted (between 7.7 and 8.4) to arrive at similar NH_3 concentrations for each total ammonia level (50 mg l^{-1} NH_4^+-N + NH_3-N: 2.2, 3.0, 4.2 mg l^{-1} NH_3-N; 100 mg l^{-1} NH_4^+-N + NH_3-N: 2.5, 3.5, 4.4, 5.5 mg l^{-1} NH_3-N; 150 mg l^{-1} NH_4^+-N + NH_3-N: 4.7 mg l^{-1} NH_3-N). Relatively small ranges of pH were chosen to prevent a direct influence of pH on the growth. Therefore, the effect of higher NH_3 concentrations could not be tested in combination with very high NH_4^+ concentrations as this would require very low and unrealistic pH values.

Data of all experiments were pooled to arrive at correlations between RGR and NH_4^+-N as well as NH_3-N. An isopleth contour map of RGR as a function of pH and total ammonia was developed using the software package SURFER (version 6 for Windows) after gridding all data (only RGR > 0) with minimum curvature (4 × 4 grid lines) and smooth spline.

3. Results

Relative growth rates (RGR) of *L. gibba* on domestic wastewater containing different combinations of ionised ammonium and un-ionised ammonia (8.26–299 mg l^{-1} NH_4^+-N + 0.03–31.4 mg l^{-1} NH_3-N) varied between 0 and 0.3 g g^{-1} DW per day. Maximum RGR was achieved with 10 mg l^{-1} total ammonia at pH 6.8, resulting in a combination of 9.97 mg l^{-1} NH_4^+-N and 0.03 mg l^{-1} NH_3-N.

At concentrations of the un-ionised form of up to 1 mg l^{-1} NH_3-N a linear correlation could be found between RGR and NH_4^+-N ($y = 0.3 - 0.0004x$, $r^2 = 0.95$, $P < 0.005$, Fig. 1) as well as NH_3-N ($y = 0.3 - 0.15x$, $r^2 = 0.96$, $P < 0.005$, Fig. 2). The linear correlation between RGR and NH_4-N disappeared at higher NH_3-N-concentrations (Fig. 1). Between 1 and 6 mg l^{-1} NH_3-N the RGR was found to be only dependent on the concentration of the un-ionised NH_3-N (mean RGR between the two levels 1–3 and 3–6 mg l^{-1} are significantly different at $P < 0.005$). This correlation was linear as well ($y = 0.28 - 0.023x$, $r^2 = 0.75$, $P < 0.005$, Fig. 2). *L. gibba* died at concentrations of

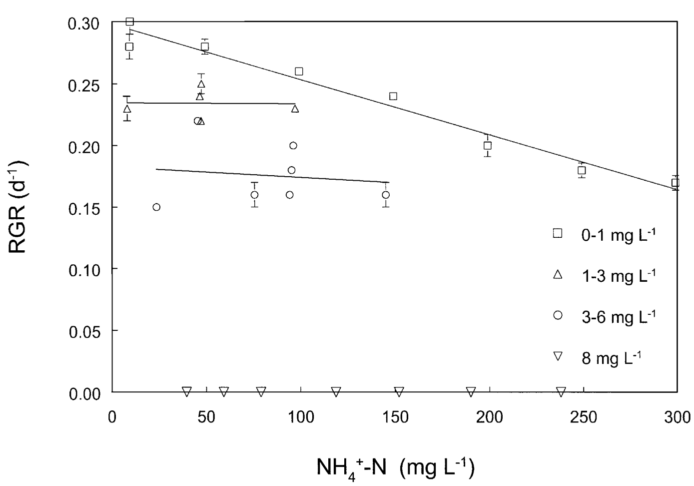

Fig. 1. Fitted curves of relative growth rates (RGR) of the duckweed *Lemna gibba* (means ± standard errors) on domestic wastewater of different ammonium (NH_4^+-N) concentrations and different levels of un-ionised ammonia (NH_3-N). At concentrations of up to 1 mg l^{-1} NH_3-N a linear correlation was found between RGR and NH_4^+-N ($y = 0.3 - 0.0004x$, $r^2 = 0.95$, $P < 0.005$) which is not significant at higher NH_3-N concentrations. Mean RGR (intercepts) between the two levels 1–3 and 3–6 mg l^{-1} are significantly different at $P < 0.005$.

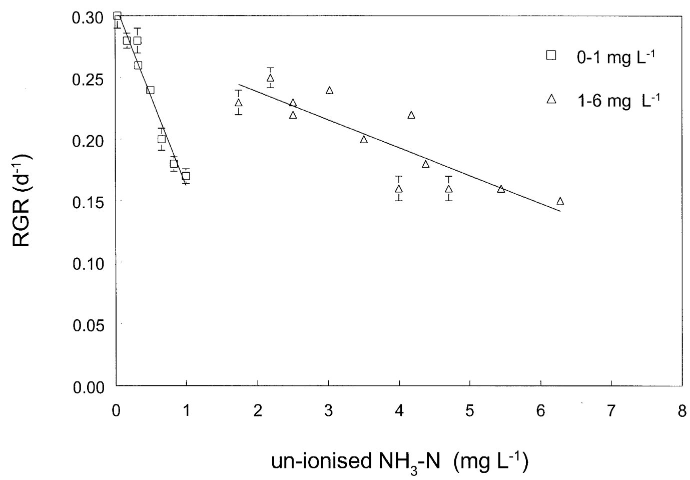

Fig. 2. Fitted curves of relative growth rates (RGR) of the duckweed *Lemna gibba* (means ± standard errors) on domestic wastewater of different un-ionised ammonia (NH_3-N) concentrations. A linear correlation was found between RGR and NH_3-N at concentrations of up to $1\,mg\,l^{-1}$ NH_3-N ($y = 0.3 - 0.15x$, $r^2 = 0.96$, $P < 0.005$) as well as for concentrations between 1 and $6\,mg\,l^{-1}$ NH_3-N ($y = 0.28 - 0.023x$, $r^2 = 0.75$, $P < 0.005$).

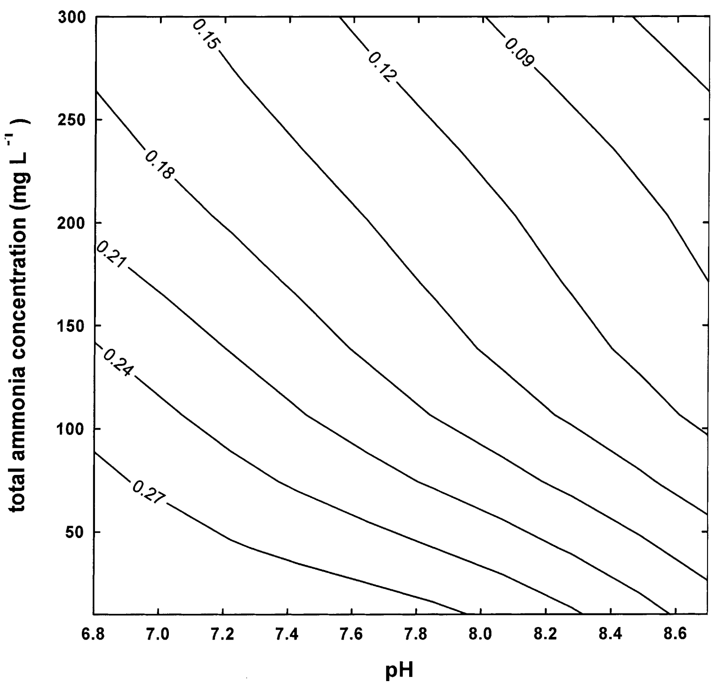

Fig. 3. Isopleth contour map of relative growth rates (in $g\,g^{-1}$ DW per day) of the duckweed *Lemna gibba* as a function of pH and total ammonia concentrations (NH_4^+-N + NH_3-N) in domestic wastewater at 23°C (y-axis starting at $10\,mg\,l^{-1}$ total ammonia).

the un-ionised form of 8 mg l^{-1} NH_3-N and higher, independent of the concentration of the ionised NH_4^+-N (Fig. 1, not shown in Fig. 2).

Using all data triples with RGR $>$ 0, the dependence of the RGR on pH and total ammonia concentrations (NH_3-N + NH_4^+-N) can be shown with contour lines after gridding (Fig. 3). Assuming 0.3 g g^{-1} DW per day to be the optimal RGR, each line represents a decrease of growth by 10% (RGR 0.27–0.09: 90–30% of optimum growth). The figure only represents a situation without limitation of growth due to low nitrogen concentrations, because experiments were starting at total ammonia concentrations of 10 mg l^{-1} (therefore, *y*-axis starts at 10 mg l^{-1} NH_3-N + NH_4^+-N). Lower concentrations are less relevant from a wastewater treatment perspective. The NH_3-N concentration above which no growth could be found (8 mg l^{-1}) corresponds to a maximum pH value of 9.9 (at 10 mg l^{-1} NH_3-N + NH_4^+-N and 23°C).

4. Discussion

For the examined range of pH, our results show that the toxicity of total ammonia on the duckweed species *L. gibba* can be attributed to the effect of only the un-ionised NH_3 at concentrations of NH_3-N higher than 1 mg l^{-1}. In this range the toxic effect of NH_4^+-N could be disregarded. The maximum tolerance level for un-ionised ammonia was detected around 8 mg NH_3-N l^{-1}. At NH_3-N concentrations below 1 mg l^{-1}, the ionised form (NH_4^+-N) contributed to the toxicity as well. A maximum tolerance level for NH_4^+ could not be detected, because this would require very low pH levels to keep NH_3 concentrations low.

A necessary assumption for these conclusions is that the relatively small pH range used (6.8–8.7) only affected the fractions of NH_4^+ and NH_3, not growth itself. Due to the given relationship Eq. (1), it is impossible to determine the toxicity of pH, NH_4^+ and NH_3 independently from each other. In practice, however, the extremes (only NH_4^+ or only NH_3) are impossible to realise. This also makes it practically impossible to test the additive effects of NH_4^+ and NH_3. Such additivity was assumed in a model for aquatic animals by Erickson (1985), but experimental evidence could not be given. A high proportion of either NH_4^+ or NH_3 can only be achieved through extreme pH values, which would affect plant growth by itself.

The highest RGR achieved during our experiments was comparable to literature values of 0.28 per day (Oron et al., 1987, Vermaat and Hanif, 1998) and can, therefore, be used as reference for uninhibited growth. Comparable values of ammonia toxicity could only be found in the study of Clement and Merlin (1995). They reported 56% inhibition of dry weight increase in *L. minor* at total ammonia concentrations of 152 mg N l^{-1} and pH adjusted at 8 (i.e. a calculated concentration of 5.7 mg l^{-1} NH_3) and no growth at concentrations of the un-ionised NH_3 above 10.5 mg l^{-1}. Lower inhibition levels were found by Caicedo et al. (2000) for *Spirodela polyrhiza*. Duckweed growth was inhibited by more than 30% for total ammonia concentrations above 50 mg l^{-1} and pH above 8. The latter two studies were the only ones using adjusted pH values, which is vital to keep the un-ionised NH_3 concentrations constant.

Our predicted contour plot (Fig. 3) suggests that *L. gibba* can be used to treat wastewater containing very high total ammonia concentrations as long as certain pH levels are not exceeded. Up to pH levels of 7.8, a substantial production of 55 kg DW ha^{-1} per day can be achieved, which is comparable to values found in full scale ponds (Alaerts et al., 1996). Wastewater treatment using *L. gibba* becomes impossible at pH levels above approximately 9.8, depending on the temperature.

As a consequence, treatment efficiencies in duckweed-covered wastewater can be increased by lowering the pH values, e.g. by anaerobic pre-treatment by means of (anaerobic) ponds or sophisticated (anaerobic) reactors (Veenstra et al., 1995). Algal growth, which increases the pH, should be prevented by maintaining a closed duckweed-cover.

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