Reprinted from
J. Aquat. Plant Manage. 25: 14-19

The Role of Egeria in Removing Nitrogen and Phosphorus from Nutrient Enriched Waters

K. R. REDDY, J. C. TUCKER, AND W. F. DEBUSK

ABSTRACT

The role of the submersed macrophyte egeria (*Elodea densa* Planch) in stripping N and P from nutrient-enriched waters was evaluated using outdoor tanks. Nitrogen and P removal rates in summer exceeded winter values by about 2-fold. Plant uptake and NH_3 volatilization were found to be the major N-removal mechanisms functioning in the system. Egeria showed a preference for NH_4^+ over NO_3^- when both ions were present in the water in equal amounts. Nitrogen and P removal rates were in the range of 186-408 mg N m^{-2} day^{-1} and 122-228 mg P m^{-2} day^{-1} respectively.

Key words: Elodea, water quality, nitrogen, phosphorus, nutrient uptake.

INTRODUCTION

In recent years, interest in the application of aquatic macrophytes for sewage treatment has increased (Stowell et al., 1981; Reddy and Sutton, 1984). Many of these systems employ either floating or emergent macrophytes. Effluent leaving floating macrophyte systems, containing plants such as water hyacinth (*Eichhornia crassipes* [Mart] Solms), is low in dissolved oxygen and usually contains unacceptable levels of P. Culturing submersed macrophytes in conjunction with floating or emergent macrophytes can aid in improving the overall water quality (Reddy et al., 1982; Reddy, 1983). In order to manage submersed macrophytes in natural or artificial systems, it is important to have a fundamental understanding of growth and nutrient uptake kinetics of these plants and their role in altering the water environment.

The study reported in this paper attempts to evaluate the capacity of one submersed macrophyte, egeria (*Elodea densa* [Planch] casp) to strip N and P from nutrient-en-

[1]Florida Agricultural Experiment Stations Journal Series No. 7023.
[2]Professor and Biological Scientists II, respectively. University of Florida, Institute of Food and Agricultural Sciences, Central Florida Research and Education Center, P. O. Box 909, Sanford, FL 32771.

riched water and its role in altering the physico-chemical environment of the water.

MATERIALS AND METHODS

Egeria used in this study was obtained from the Wekiva River located near Sanford, Florida. A description of the individual experiments is as follows:

Experiment I

This study was conducted to determine the growth characteristics of egeria cultured under non-limiting nutrient conditions using duplicate 1000 l outdoor concrete tanks (1.7 m^2 surface area; measuring 222 cm long, 77 cm wide and 52 cm deep). Two Vexar mesh baskets (0.25 m^2 surface area and 53 cm depth) containing 175 g (dw) m^{-2} of egeria were placed in each tank to monitor growth rates. Since there was no sediment in the concrete tanks, plants were freely suspended in the water column. Plants were maintained at the same density inside and outside the baskets. At the end of each week, Vexar mesh baskets were removed from the tanks, allowed to drain for 5 min., weighed, and returned to the respective tanks. Once maximum density was reached and no net growth was recorded, plants were harvested to their original density.

Both tanks were filled with 900 l of nutrient medium containing NH_4-N = 10.5 mg l^{-1}, NO_3-N = 10.5 mg l^{-1} PO_4-P = 3.0 mg l^{-1}, K = 23 mg l^{-1}, Ca = 70 mg l^{-1}, Mg = 20 mg l^{-1}, Fe-EDTA = 0.6 mg l^{-1}, and micronutrients. This composition was chosen to simulate the nutrient concentrations commonly observed in sewage effluents. Micronutrients were applied through a commercially available liquid fertilizer (Nutrispray—Sunniland, Chase & Co., Sanford, Florida). The nutrient medium in the tank was mixed by submersible pumps which operated on a 12-hr per day cycle. Once a week, water in each vault was replaced with fresh medium containing the above described chemical composition.

Water samples (duplicate) were obtained once a week and analyzed for N and P forms. Extensive water sampling was performed during the summer of 1982 (June-September) and the winter of 1982-83 (December-February). During this period, water samples (duplicate) were collected at 0, 1, 2, 4, and 7 days and analyzed for NH_4^+, NO_3^- and ortho-P (A.P.H.A., 1985). Plant samples were obtained once every 2 weeks during summer and winter, dried at 70C, ground to pass through a 20 mesh sieve, and analyzed for N and P (Bremner and Mulvaney, 1982; Jackson, 1965).

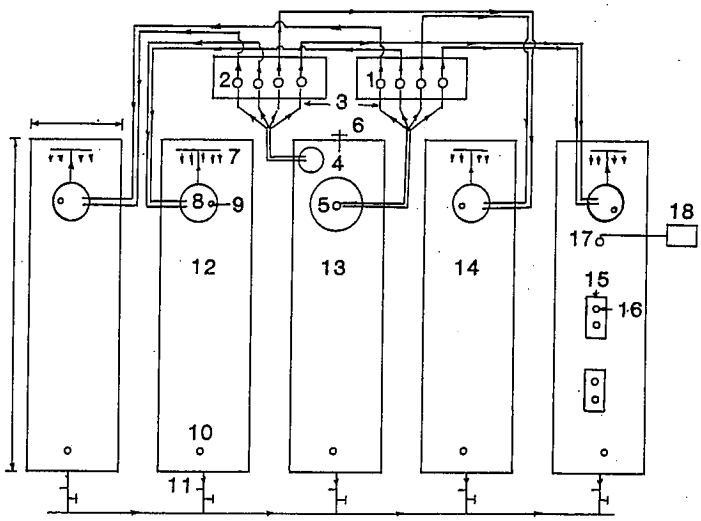

Figure 1. Schematic presentation of the flow-through system. Arrows indicate the direction of water flow. 1) Peristaltic pumps for nutrient solution, 2) peristaltic pumps for diluent (well water), 3) tygon pump tubing, 4) well-water filter, 5) nutrient solution reservoir, 6) inflow value for well water, 7) inflow of water enriched with nutrients, 8) nutrient-well water mixing cylinder, 9) submersible pump, 10) overflow, 11) outflow sampling port, 12) tanks with 30 cm sediment and 40 cm overlying water, 13) well-water reservoir (covered), 14) tanks with no sediment and 40 cm water column, 15) floating plexiglass chamber, 16) 1 *N* H_2SO_4 traps, 17) oxygen probe, 18) recorder attached to oxygen probe.

Experiment II

This study was conducted to determine the preferential uptake of N by egeria when supplied with equal amounts of NH_4^+ and NO_3^-. There were three replications for each of two treatments. One treatment contained $^{15}NH_4NO_3$, while the second treatment contained $NH_4^{15}NO_3$. Ten grams of egeria (fw) were grown in one liter containers placed in an environmentally controlled room at 20C. Artificial light was provided for 14 hrs day^{-1} at an intensity of 200 $\mu E\ m^{-2}\ s^{-1}$. Each container was enriched with 12.5 mg N l^{-1} (NH_4^+-N and NO_3^--N in equal proportions added as NH_4NO_3), 3.1 mg P l^{-1}, and secondary and micronutrients as described in Experiment I. Plants were exposed to nutrient solutions for 0, 1, 2, 4, 8, 24, 48, 96 and 144 hrs and removed from the solutions, rinsed in deionized water, dried at 70C for a period of 48 hours, and dry weights recorded. Plant samples were ground to pass through a 20 mesh sieve and analyzed for TKN and labeled N content. During the same sampling period, water

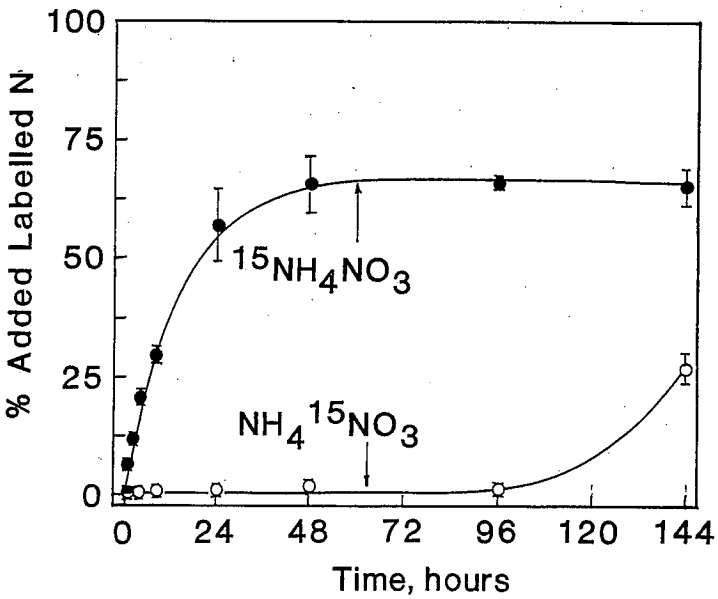

Figure 2. Percent of added labeled N recovered in plant tissue of *E. densa*. Nitrogen was added as NH_4NO_3, and plants were cultured at 20C in environmentally controlled growth chambers.

samples from each container were also obtained and analyzed for NH_4^+ and NO_3^- content using standard methods. At the end of 144 hours, 1 ml H_2SO_4 was added to the water and the volume was decreased to 100 ml by evaporating the samples at 70C. Water samples were analyzed for NH_4^+ and NO_3^- by steam distillation and subsequently for labeled-N content.

Experiment III

This experiment was designed to measure the nutrient-removal rates of egeria cultured in experimental tanks equipped with a continuous flow of nutrients at varying loading rates. A schematic presentation of the experimental setup is shown in Fig. 1. Egeria was placed in concrete tanks (surface area = 1.7 m^2), at a density of 1.5 kg (fw) m^{-2}. The tanks were elevated by 5 cm on the inflow end to insure constant flow of nutrient solution toward an overflow pipe located at the low end of the tank. There were two treatments with two replications of each treatment.

One treatment had a 12 cm layer of bottom sediment (organic Histosol) while the other treatment had no bottom sediment. Both treatments contained about 680 l (40 cm depth) of nutrient solution flowing through the systems at a rate of about 315 ml min^{-1}, equivalent to a retention time of 36 hr. A Masterflex pump with four pump heads supplied artesian well water (310 ml min^{-1}) to mixing cylinders located in each of the four experimental tanks. Another Masterflex pump supplied nutrient solution (5 ml min^{-1}) to each of the mixing cylinders. The mixing cylinders were made from PVC pipe (15 cm diameter by 92 cm height) capped at both ends. These cylinders were placed vertically at the elevated end of each tank. Both nutrient solution and well water were injected through separate glass tubes to the bottom of the cylinder, where a submersible pump insured complete mixing of the two solutions. An overflow pipe at a height of 66 cm channeled the diluted solution into the tank to a point 23 cm below the surface. The solution flowed through the tank to an outflow pipe where it was removed from the system. A diverter valve located on the outflow pipe allowed for sample collection of the outflow.

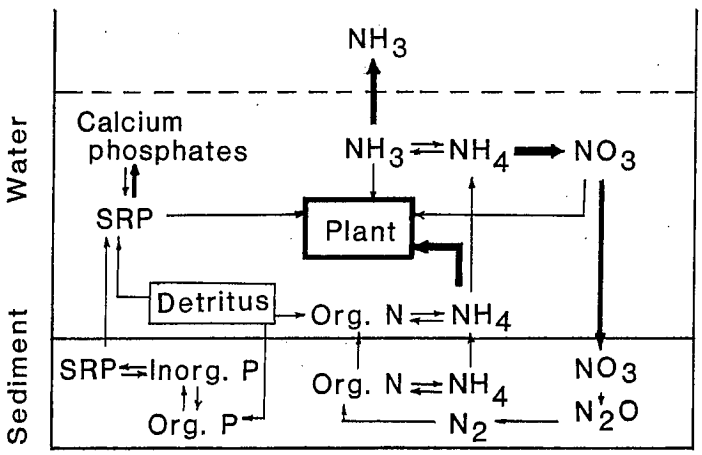

Figure 3. Schematic presentation of the biochemical and physico-chemical processes functioning in sediment-water column containing egeria.

After a 10-day equilibration period at low nutrient level, the N and P concentrations were varied once a week for each of the next three weeks (July-August, 1985), at levels of 1, 2, and 4 mg N l^{-1}, respectively, and 0.2, 0.4 and 0.8 mg P l^{-1}, respectively. Nitrogen and P were added as ammonium chloride and potassium phosphate, respectively.

The tanks were subdivided into three evenly spaced sections and plant weights were taken from each section every seven days. Egeria was then restocked at the original starting density (1.5 kg[fw] wt m^{-2}). Plant samples were also taken from these three sections at 0 and 7 days of each week, dried at 70°C for 48 hrs and dry weights determined. Water samples were taken twice during each week at the inflow, the outflow, and from each of the three sections along the length of the tank. Plant samples were analyzed for TP and TKN. Water samples were analyzed for NH_4^+-N and NO_3^--N, and ortho-P, using standard methods (A.P.H.A., 1985).

An airtight plexiglass box capable of floating on the water surface of each tank was used in an effort to collect NH_3 gas evolved from the system. Each box had two beakers containing 50 ml of 1 *N* H_2SO_4 suspended about 4 cm above the water surface. Acid traps were left in place for 72 hrs and analyzed for NH_4-N content on an AutoAnalyzer using standard methods (A.P.H.A., 1985).

RESULTS

Growth and Nutrient Removal

Egeria showed minimal seasonal effect on biomass yields. Growth rates (2.5-3.8 g (dw) m^{-2} day^{-1}, Table 1) of egeria were linear within a plant density range of 175-410 g (dw) m^{-2}. Average plant tissue N was 35.6 ± 3.1 g kg^{-1} for summer and 40.3 ± 4.7 g kg^{-1} for winter respectively. During the same period, P content of the tissue was 13.7 ± 1.7 g kg^{-1} for summer and 12.8 ± 1.6 g kg^{-1} for winter.

Ammonium N was rapidly lost from water in both summer and winter, with levels decreasing to negligible concentration within 7 days. A decrease in NH_4^+ corresponded to an increase in NO_3^- levels. Phosphorus loss from the water showed a distinct seasonal effect, with removal more rapid during summer than winter. Rate of N and P loss from water showed an exponential decrease and followed the relationship shown in the equation below:

$$C_t = C_o\,[1\text{-}\exp(-kt)]$$

TABLE 1. GROWTH RATES OF *ELODEA DENSA* UNDER NUTRIENT NON-LIMITING CONDITIONS. B_t = BIOMASS (dw) m^{-2} AT TIME = t DAYS; GR = GROWTH RATE, g (dw) m^{-2} DAY^{-1}; B_o = BIOMASS (dw) m^{-2} AT t=0 DAYS.

Growing season	$B_t = GR.t + B_o$	R^2	n
Winter 12/30/81-3/15/82	$B_t = 3.55\,t + 176.4$	0.99**	48
Spring 3/15/82-6/14/82	$B_t = 3.81\,t + 183.4$	0.99*	56
Summer 6/14/82-9/20/82	$B_t = 2.51\,t + 188.8$	0.90**	60
Fall 9/20/82-11/29/82	$B_t = 3.61\,t + 180.7$	0.99*	44

where C_t = inorganic N or P loss from water; C_o = initial N or P concentration of the water; k = removal rate coefficient; and t = residence time. Using a least-square fit of the data, N and P removal rate coefficients were calculated (Table 2). Nitrogen and P removal coefficients for summer were about twice those calculated for winter months. The rate coefficients reported in Table 2 indicate that it would require about 46 days to reduce the N concentration by 50% during winter months, and 153 days to reduce N concentration by about 90%.

Data on mass balance of N are shown in Table 3. During summer about 85% of the N added was accounted for either in water or in the plant tissue, while 97% was accounted for during winter months. All of the added N was recovered as NO_3^- at the end of 7 days. Plant uptake accounted for 6.5-6.9% of recovery, indicating poor efficiency of egeria in assimilating N. Plants were found to be slightly more efficient in assimilating P (16-21% of added P) (Table 3). About 34-63% of the added P was not accounted for in either the plant tissue or in the water. Less P was lost during winter than summer.

Preferential Plant Uptake of Inorganic N Ions

Results of the growth chamber study indicate that egeria prefers NH_4^+ over NO_3^- when both ions are supplied to the plant in equal proportions (Fig. 2). Assimilation of $^{15}NO_3^-$ by plant tissue was observed only after 96 hours, at which point NH_4^+ levels in the water had decreased to negligible levels. About 64% of the added $^{15}NH_4^+$ was recovered in the plant tissue within 48 hours, whereas less than 0.1% of the added $^{15}NO_3^-$ appeared in the plant. At the end of 144 hrs, however, about 25% of the added $^{15}NO_3^-$ was recovered in the plant tissue.

TABLE 2. NITROGEN AND PHOSPHORUS REMOVAL COEFFICIENT (k DAY^{-1}) FROM WATER CONTAINING *ELODEA DENSA*. RATE COEFFICIENTS ARE THE AVERAGES OF 20 RUNS DURING SUMMER AND 18 RUNS DURING WINTER. VALUES SHOWN IN PARENTHESES ARE THE NUMBER OF DAYS REQUIRED FOR 50% REMOVAL OF N AND P FROM THE WATER.

Season	Nitrogen	Phosphorus
Summer	0.038 (18.2)	0.258 (2.7)
Winter	0.015 (46.2)	0.100 (6.9)
L.S.D. 0.05	0.018	0.032

TABLE 3. MASS BALANCE OF NITROGEN AND PHOSPHORUS (mg m^{-2} DAY^{-1}) IN EXPERIMENTAL TANKS CONTAINING *ELODEA DENSA* AND MAINTAINED AT 7-DAY RESIDENCE TIME WITH NUTRIENT-ENRICHED WATER. SUMMER = JUNE-SEPTEMBER; WINTER = DECEMBER-FEBRUARY. VALUES IN PARENTHESES ARE THE PERCENT OF ADDED N OR P, RESPECTIVELY.

Nutrient-fraction	Summer	Winter
Nitrogen:		
N added	1860	1975
Plant uptake	129 (6.9)	129 (6.5)
N remaining in the water	1452 (78.1)	1789 (90.6)
Total N recovered	1581 (85.0)	1918 (97.1)
N unaccounted for	279 (15.0)	57 (2.9)
Phosphorus:		
P added	287	221
Plant uptake	46 (16.0)	46 (20.8)
P remaining in the water	59 (20.6)	99 (44.8)
Total P recovered	105 (36.6)	145 (65.6)
P unaccounted for	182 (63.4)	76 (34.4)

Effect of N and P Concentration of Water on Growth and Nutrient Removal

Nitrogen and P concentrations of the water at the inflow and outflow of experimental tanks containing egeria are shown in Table 4. The experimental system was loaded with NH_4^+ at varying rates with a residence time of 36 hours. Both inflow and outflow water contained primarily NH_4^+ with negligible concentrations of NO_3^- (< 0.01 mg N l^{-1}). At three nutrient loading rates, N concentration of the inflow was reduced by about 94% in 36 hours. These data are based on one-week duplicate runs at each nutrient loading. The presence of sediment did not influence N removal from the water column. At all levels of P loading, outflow P concentration was slightly higher in the tanks containing sediment than in the tanks with no sediment. Reduction in P concentration was found to be in the range of 12-65% in the tanks with sediment, as compared to 68-83% in the tanks without sediment.

Mass balance at various N loadings indicate that N removal due to plant assimilation was in the range of 152-309 mg N m^{-2} day (Table 5). Nitrogen uptake was slightly higher by the plants cultured in the tanks with underlying sediment, as compared to the plants grown with no underlying sediment. At all N-loading rates, about 44-69% of the added N was unaccounted for, for all fractions measured. Nitrogen loss due to NH_3 volatilization accounted for only 0.6-2.8% of the added NH_4-N.

Phosphorus removal due to plant uptake was in the range of 24-32 mg P m^{-2} day^{-1} (Table 6). At all P loading rates, about 15-56% of the added P was not accounted for. Phosphorus recovery in the water and plant tissue was higher in the treatment with underlying sediment than in the system with no sediment.

DISCUSSION

The results of this study indicate that submersed aquatic macrophytes can be potentially used to strip N and P from nutrient-rich waters. Although nutrient removal by egeria was equal to or greater than that by many macrophytes (Reddy and DeBusk, 1985), plant uptake appears

TABLE 4. AMMONIUM-N AND ORTHO-P CONCENTRATIONS (mg l^{-1}) OF THE WATER AT INFLOW AND OUTFLOW OF THE TANKS CONTAINING *ELODEA DENSA*. EACH VALUE IS AN AVERAGE OF 6 SAMPLES.

	Nitrogen		Phosphorus	
Treatment	Inflow	Outflow	Inflow	Outflow
Tanks with sediment	1.23 ± 0.20	0.06 ± 0.01	0.18 ± 0.02	0.16 ± 0.03
	2.02 ± 0.41	0.10 ± 0.06	0.41 ± 0.05	0.12 ± 0.05
	3.95 ± 0.75	0.25 ± 0.03	0.71 ± 0.17	0.25 ± 0.09
Tanks without sediment	1.24 ± 0.19	0.08 ± 0.02	0.23 ± 0.09	0.04 ± 0.03
	2.31 ± 0.46	0.10 ± 0.02	0.46 ± 0.10	0.08 ± 0.06
	3.75 ± 0.69	0.24 ± 0.07	0.72 ± 0.15	0.23 ± 0.07

TABLE 5. MASS BALANCE OF NITROGEN IN EXPERIMENTAL TANKS CONTAINING *ELODEA DENSA* AND OPERATED AT 1.5 DAY RESIDENCE TIME WITH 3 LEVELS OF NUTRIENT MEDIUM. NUTRIENT LEVEL I, II, AND III CONSISTED OF 1, 2, AND 4 mg N l^{-1} AND 0.2, 0.4, AND 0.8 mg P l^{-1}, RESPECTIVELY. VALUES SHOWN IN PARENTHESES ARE PERCENT OF ADDED N.

N-fraction	Tanks with sediment (mg N m^{-2} day^{-1})	Tanks without sediment (mg N m^{-2} day^{-1})
Nutrient Level I		
N added	328	331
Plant upake	167 (50.9)	152 (45.9)
N remaining in the water	16 (4.9)	21 (6.3)
Total N recovered	183 (55.8)	173 (52.2)
N unaccounted for	145 (44.2)	158 (47.8)
Nutrient Level II		
N added	539	616
Plant uptake	225 (41.7)	225 (36.5)
N remaining in the water	27 (5.0)	27 (4.4)
Total N recovered	252 (46.8)	252 (40.9)
N unaccounted for	287 (53.2)	364 (59.1)
Nutrient Level III		
N added	1053	1000
Plant uptake	309 (29.3)	149 (14.9)
N remaining in the water	67 (6.4)	64 (6.4)
Total N recovered	376 (35.7)	213 (21.3)
N unaccounted for	677 (64.3)	787 (78.7)

TABLE 6. MASS BALANCE OF PHOSPHORUS IN EXPERIMENTAL TANKS CONTAINING *ELODEA DENSA* AND OPERATED AT 1.5 DAY RESIDENCE TIME WITH 3 LEVELS OF NUTRIENT MEDIUM. NUTRIENT LEVEL I, II, AND III CONSISTED OF 1, 2, AND 4 mg N l^{-1} AND 0.2, 0.4, AND 0.8 mg P l^{-1}, RESPECTIVELY. VALUES IN PARENTHESES ARE PERCENT OF ADDED P.

P-fraction	Tanks with sediment (mg P m^{-2} day^{-1})	Tanks without sediment (mg P m^{-2} day^{-1})
Nutrient Level I		
P added	48	61
Plant upake	27 (56.3)	32 (52.5)
P remaining in the water	43 (89.6)	11 (18.0)
Total P recovered	70 (145.8)	43 (70.5)
P unaccounted for	+22 (+45.8)	18 (29.5)
Nutrient Level II		
P added	109	123
Plant uptake	25 (22.9)	23 (18.7)
P remaining in the water	32 (29.4)	21 (17.1)
Total P recovered	57 (52.3)	44 (35.8)
P unaccounted for	52 (47.7)	79 (64.2)
Nutrient Level III		
P added	189	192
Plant uptake	24 (12.7)	29 (15.1)
P remaining in the water	63 (33.3)	61 (31.8)
Total P recovered	87 (46.0)	90 (46.9)
P unaccounted for	102 (54.0)	102 (53.1)

to play a poor role in stripping nutrients. However, the capacity of these plants to elevate pH and dissolved O_2 appears to play a significant role in stripping nutrients from water. Submersed macrophytes are usually less efficient in trapping solar energy than many of the floating or emergent macrophytes (Garrard and Van, 1982; Reddy et al., 1983), primarily because of poor light transmission through water, slow rate of CO_2 diffusion through water, relatively low activities of carboxylation enzymes, and low light-saturation points (Van et al., 1976; Bowes et al., 1979; Anton Hough, 1979; Reddy et al., 1983). Because of poor growth rates of egeria, harvesting the biomass for by-product utilization may not be economically feasible. However, these plants can be cultured in overlying waters of emergent macrophyte systems (Reddy, 1983) or in ponds connected to floating macrophyte ponds used for water treatment (Reddy et al., 1982).

Although N and P removal due to plant uptake was the same both in winter and summer, overall nutrient removal was significantly higher in summer than in winter. This is expected because both N and P transformations are affected by water temperature. Nitrification was found to be very active in the batch-feed system (7 day residence time, Experiment I), where loss of NH_4^+ was correlated with the increase in NO_3^- levels of the water. Nitrogen recovery at the end of 7 days was 85 to 97% of the added NH_4NO_3, with 3-15% of the added N lost from the system. This loss could be due to NH_3 volatilization, because of high pH conditions that occurred during the day when photosynthetic activity of egeria was high (Bouldin et al., 1974; Mikkelsen et al., 1978). Plants appear to prefer NH_4^+ ions over NO_3^- ions, as was demonstrated in the tracer study (Fig. 2). Rapid oxidation of NH_4^+ to NO_3^- resulted in poor removal of N from the system. Under natural conditions, NO_3^- formed during the oxidation of NH_4^+ can be potentially lost from the system, because NO_3^- diffuses into the underlying sediments where it can be denitrified to gaseous end products. In our study, absence of sediment in experimental tanks (Experiment 1) resulted in accumulation of NO_3^- in the water. The biochemical and physicochemical processes functioning in sediment-water column containing egeria are schematically presented in Fig. 3. Some of these processes were evaluated in this experiment.

Rapid removal of N was observed when NH_4^+ was the sole source of N and was added at low concentrations. Plant uptake resulted in removing 25-50% of the added N, while overall N removal was on the order of 55-90%. The major loss mechanism appears to be NH_3 volatilization, since no significant concentrations of NO_3^- were observed. At low N concentrations (Experiment III) plants produced greater amounts of biomass (data not shown) than were observed at higher N additions (Experiment I), thus resulting in larger N recovery in the plant tissue (Table 5).

Maximum plant uptake of P (46 mg P m^{-2} day^{-1}) was observed when loading rate was 221-287 mg P m^{-2} day^{-1}. At low P loading rates a greater proportion of added P was removed by the plant (53-56%). However, overall P removal by the system was on the order of 30-70%, indicating the functioning of other processes in the system. High pH conditions in the water probably resulted in precipitation of soluble P with calcium, forming insoluble calcium phosphates. In our experiment, uptake of P by algae was not determined, and this uptake can also contribute to the sum of the unaccounted-for P.

In conclusion, this study has shown that the submersed macrophyte egeria has a potential use in stripping N and P from nutrient enriched water. This plant has greater applicability in wastewaters with low levels of N (< 4 mg

N l^{-1}) and P (< 1 mg P l^{-1}), since plants grow better at these levels of enrichment. Nitrogen removal rates were 186 and 408 mg N m^{-2} day during winter and summer months, respectively, while P removal rates were 122 and 228 mg P m^{-2} day^{-1}, respectively.

ACKNOWLEDGEMENTS

This paper is a contribution of a cooperative program between the Institute of Food and Agricultural Science (IFAS) of the University of Florida and the Gas Research Institute (GRI), entitled, "Methane from Biomass and Waste."

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