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PRODUCTION AND NUTRIENT REMOVAL BY PERIPHYTON GROWN UNDER DIFFERENT LOADING RATES OF ANAEROBICALLY DIGESTED FLUSHED DAIRY MANURE[1]

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Growing algae to scrub nutrients from manure presents an alternative to the current practice of land application and provides utilizable algal biomass as an end product. The objective of this study was to assess algal growth, nutrient removal, and nitrification using higher light intensities and manure loading rates than in the previous experiments. Algal turfs, with periphyton mainly composed of green algal species, were grown under two light regimes (270 and 390 μmol photons · m^{-2} · s^{-1}) and anaerobically digested flushed dairy manure wastewater (ADFDMW) loading rates ranging from 0.8 to 3.7 g total N and 0.12 to 0.58 g total P · m^{-2} · d^{-1}. Filamentous cyanobacteria (*Oscillatoria* spp.) and diatoms (*Navicula*, *Nitzschia*, and *Cyclotella* sp.) partially replaced the filamentous green algae at relatively high ADFDMW loading rates and more prominently under low incident light. Mean algal production increased with loading rate and irradiance from 7.6 ± 2.71 to 19.1 ± 2.73 g dry weight · m^{-2} · d^{-1}. The N and P content of algal biomass generally increased with loading rate and ranged from 2.9%–7.3% and 0.5%–1.3% (by weight), respectively. Carbon content remained relatively constant at all loading rates (42%–47%). The maximum removal rates of N and P per unit algal biomass were 70 and 13 mg · g^{-1} dry weight · m^{-2} · d^{-1}, respectively. Recovery of nutrients in harvested algal biomass accounted for about 31%–52% for N and 30%–59% for P. Recovery of P appeared to be uncoupled with N at higher loading rates, suggesting that algal potential for accumulation of P may have already been saturated. It appears that higher irradiance level enhancing algal growth was the overriding factor in controlling nitrification in the algal turf scrubber units.

Key index words: **agriculture; algal turf scrubbers; carbon; flushed dairy manure; livestock; nitrification; nutrient content; nutrient removal; periphyton; phytoremediation**

Abbreviations: **ADFDMW, anaerobically digested flushed dairy manure wastewater; ATS, algal turf scrubbers; DW, dry weight; SRP, soluble reactive phosphorus; TKN, total Kjeldahl nitrogen; TN, total nitrogen; TP, total phosphorus**

Controlling the input of nitrogen (N) and phosphorus (P) from non-point sources such as agricultural runoff and livestock manures into aquatic systems and the atmosphere poses a challenge in environmental protection (Adey et al. 1993, Van Horn et al. 1994, Kaiser 2001). During storage and land application of manure, large amounts of N are lost to the atmosphere due to volatilization of ammonia (Van Horn et al. 1994, Kaiser 2001). In addition, manure nutrients can be transported offsite in runoff and by leaching through the soil (Van Horn et al. 1994). Ecologically sound manure management on farms is vital to control such environmental hazards. Growing algae to scrub the nutrients from manure presents an alternative to the current practice of land application and provides utilizable algal biomass as an end product (Baumgarten et al. 1999, Mulbry and Wilkie 2001, Wilkie and Mulbry 2002).

The use of algae for improving water quality (pH, dissolved oxygen, suspended solids, etc.) and removal of nutrients and metals from eutrophic or contaminated water has been increasing over the past few decades (Oswald 1988, Craggs et al. 1996, Troell et al. 1999). Treatment of municipal, industrial, and agricultural wastewater in high rate algal ponds using mixed or monocultures of suspended algae was documented by Hoffmann (1998). High rates of nutrient removal and algal production have been measured with monocultures of cyanobacteria such as *Spirulina* (*Arthrospira*) (Lincoln et al. 1996, Olguin et al. 1997) and *Phormidium* (Blier et al. 1996) grown on manure effluent from dairy and swine operations. The ease of harvest in treatment systems using attached algae or periphyton is a major advantage over systems with suspended algae (Hoffmann 1998). However, application of periphyton in phytoremediation is a less common practice in wastewater treatment (Hoffmann 1998) and is little known, especially in dairy waste. Previously, removal of nutrients from raw and anaerobically digested dairy manure using attached algae was studied in laboratory-scale algal turf scrubber (ATS)

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units (Mulbry and Wilkie 2001, Wilkie and Mulbry 2002). Under loading rates of 0.5 to 1 g total nitrogen (TN) and 0.07 to 0.15 g total phosphorus (TP) $\cdot$ $m^{-2} \cdot d^{-1}$, algal production as dry weight (DW) was about $5\,g \cdot m^{-2} \cdot d^{-1}$. Harvested biomass contained 5%–7% N and 1.5%–2% P and accounted for 33%–42% of input TN and 58%–100% of TP. However, high nitrification rates led to high nitrate levels in treated effluent of the digested manures. Considering higher algal production and nutrient removal rates reported for similar scrubbing systems treating agricultural runoff and municipal wastewater (Adey et al. 1993, Craggs et al. 1996), it was apparent that algal growth potential was not optimized for the dairy manures studied. Light was a likely factor limiting algal growth because the average incident light on the scrubbers was only 40–140 μmol photons $\cdot m^{-2} \cdot s^{-1}$. The objective of the present study was to assess algal growth, nutrient removal, and nitrification using higher light intensities and manure loading rates, covering and exceeding the range used in the previous experiments.

MATERIALS AND METHODS

Dairy manure and ATSs. The experiments were run on anaerobically digested flushed dairy manure wastewater (ADFDMW) from the Dairy Research Unit of the University of Florida in Gainesville, Florida, USA. The flushed dairy manure underwent mechanical solids separation and settling prior to being pumped into a 400 m3 fixed-film anaerobic digestor operating at ambient temperature and a 2-day hydraulic retention time (Wilkie 2003). All tests were performed on one batch of ADFDMW that was shipped to Maryland in 200-L barrels and stored at 4° C. Aliquots of ADFDMW were dispensed after thorough mixing of large containers. The ADFDMW concentrations of ammonium (NH_4-N), nitrate (NO_3-N), soluble reactive P (SRP), TN, and TP were 233, <1, 55.8, 412, and 64.5 $mg \cdot L^{-1}$, respectively. Three ATS units each containing 1-m^2 growing area were operated in a semicontinuous mode by recycling 208 L of effluent contained in plastic drums and adding 2–9 L of ADFDMW daily. Details of the setup and operation of the ATS units have been previously described (Mulbry and Wilkie 2001, Wilkie and Mulbry 2002). The units were maintained at ambient laboratory temperature (19–24° C) and illuminated by two 400-W metal halide lights under nearly continuous light (23-h photoperiod). Incident light averaged about 270 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (range, 160–460) on two scrubbers and 390 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (range, 240–633) on a third unit, which are referred to as "low" and "high" light, respectively, in further discussion. To minimize volatilization of ammonia, pH of the effluent was maintained between 7 and 7.5 by bubbling the system with carbon dioxide.

Four different loading rates of ADFDMW (2, 4, 6, and 9 $L \cdot d^{-1}$), ranging from 0.8 to 3.7 g TN and 0.13 to 0.58 g TP $\cdot m^{-2} \cdot d^{-1}$, were tested. Algal biomass was harvested every 3–7 days by removing the screens from the ATS unit and scraping off the biomass with a rigid plastic ruler. More frequent harvest (every 3 days) was required for the high loading rates because overgrowth of turfs leads to breaking, sloughing off, and loss of biomass from the screen into the effluent. Biomass was drained on a 1-mm sieve and dried at 70° C in a heat-resistant glass dish. Ash-free DW was determined after burning dried samples at 500° C for 5 h. Liquid passing through the sieve, referred to as "brown water" and analyzed for biomass and nutrient content in Adey et al. (1993), was not routinely collected. Algal biomass estimates were based only on drained harvest retained on the sieve. After each harvest, about half of the effluent (100 L) was replaced with deionized water to avoid accumulation of salts and metabolites, as well as increases in turbidity from broken fragments of algae and inorganic particulates. Grazer populations (especially chironomid larvae) were controlled by adding 20 mL of *Bacillus thuringiensis* larvicide ("Aquabac-xt," Becker Microbial Products, Plantation, FL, USA) to the effluent (0.01% concentration) after harvest.

Chemical analyses. Harvested and dried biomass was analyzed for total Kjeldahl nitrogen (TKN) and TP after acid digestion (USEPA 1979). Total nitrogen was determined as the sum of TKN and NO_3-N. Elemental carbon (C) and nitrogen were analyzed on dried samples with a CHN analyzer (Perkin-Elmer 2400, Norwalk, CT, USA). The ATS effluent samples were taken before and after change of effluent at the end of a harvest cycle and stored at 4° C. Effluent and ADFDMW samples were analyzed for ammonia with the Nessler reaction and for SRP with the ascorbic acid method (APHA 1995). Color reactions were conducted in 96-well microplates, and absorbance was read on a microplate reader, similar to methods applied in D'Angelo et al. (2001) for soil extracts. Nitrate and nitrite were measured with an ion chromatography using IC-Pak A column and UV detector (Waters, Inc., Milford, MA, USA). The TN and TP in effluent and ADFDMW were determined as NO_3-N and PO_4-P after alkaline persulfate digestion of appropriately diluted samples (D'Elia et al. 1977, Valderrama 1981). Values for TN and TP from liquid digests were very similar to results from TKN and TP analyses of freeze-dried manure that were determined by flow injection analysis (Lachat Instruments, Milwaukee, WI, USA) as NH_4-N and PO_4-P after acid persulfate digestion. For each loading rate, five to nine harvests of established turfs were used to calculate production rate, nutrient content, and removal efficiency.

Statistical analyses. The variables were modeled as two-factor general linear models where the effects of nutrient load and incident light levels were analyzed. One-way analysis of variance (ANOVA) was used to include the treatment with low light at a loading rate of 2 $L \cdot d^{-1}$ for which there was no parallel treatment at high light. The assumptions of the general linear model were checked for each variable before ANOVA, and variance heterogeneity was corrected for means comparisons. Back-transformed values are reported in the tables and figures. The statistical analyses were performed using SAS/STAT, version 8 (1999, SAS Institute Inc., Cary, NC, USA).

RESULTS

Scrubber community. The ATS units, originally established with algal consortia from a nearby stream in Beltsville, Maryland, USA were dominated by filamentous green algae, including *Microspora willeana* Lagerh. (most abundant species on the screens), *Ulothrix ozonata* (Weber and Mohr) Kütz., *Rhizoclonium hieroglyphicum* (C.A. Agardh) Kütz., and *Oedogonium* sp. (Mulbry and Wilkie 2001). The same composition was generally true for the present study. Qualitative microscopic examination was made on the algal composition of samples taken from different sections of the algal turf (surface, inner, and underside) during harvest. Differences were observed at higher loading rates, where bright green turfs with long hairy filaments were partially replaced by dark colored patches of filamentous cyanobacteria, mainly *Oscillatoria* spp., and diatoms, including species of *Navicula*, *Nitzschia*, and *Cyclotella*. This change in composition was prominent with low light (270 μmol photons · m^{-2} · s^{-1}) at a loading rate of 3.7 g TN and 0.58 g TP · m^{-2} · d^{-1} (9 L manure · d^{-1}) but was not so apparent under high light (390 μmol photons · m^{-2} · s^{-1}). Marked changes in algal composition were observed with high light at a higher ADFDMW loading rate of 4.9 g TN and 0.77 g TP · m^{-2} · d^{-1} (12 L · d^{-1}), where turfs with green filamentous algae failed to grow and were replaced by dark patches of cyanobacteria and diatoms. No further experiments were conducted at that rate. The most common planktonic forms in the effluent were green algae, including *Scenedesmus* sp., *Chlorella* sp., and *Tetraedron minimum* (A.Br.) Hansg. *Oscillatoria* spp. were the most common cyanobacteria, but heterocystous filaments of *Anabaena* spp. were more frequently observed at the lowest loading rate.

The most common macroscopic grazers of the ATS units were the midge larvae (*Chironomus*) that occasionally attained high abundance, especially on the sides of the scrubber area and on the screens. Ciliates, nonpigmented flagellates, rotifers (*Philodina*?), and oligochaetes (*Aeolosoma*?) were common in the effluent. Limpets were common inhabitants of the plastic drums containing the effluent, where they grew on the walls of the drum and on the water pump. Grazer activity did not appear to be important in reducing algal biomass, but some unexplained variations in biomass may partially be attributed to variations in grazer pressure. Short harvest interval and constant disturbance in ATS were believed to prevent grazer populations from building up to significant levels (Adey and Loveland 1998). In our experience, addition of larvicide to the effluent was effective in curbing the larval population. The chironomid larvae get imbedded in and on the turf as well as on the sides of the scrubber area. Flushing the turf screens with water pressure and thorough cleaning of the scrubber area after harvest decreased the larval population, but the larvicide was more effective, particularly at the high ADFDMW loading rates.

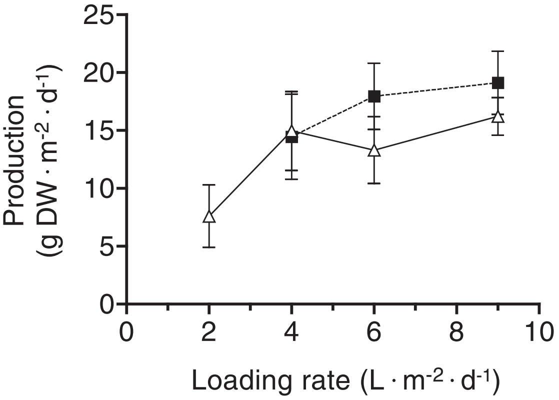

Fig. 1. Algal production rate (g DW · m^{-2} · d^{-1}) under different loading rates of anaerobically digested flushed dairy manure wastewater and incident light (triangles, 270 μmol photons · m^{-2} · s^{-1}; squares, 390 μmol photons · m^{-2} · s^{-1}). Data points represent mean values of five to nine measurements, and error bars are SDs.

Algal production. Production generally increased with ADFDMW loading rate (Fig. 1). It ranged from 5 to 23 g DW · m^{-2} · d^{-1} with mean values of 7.6 ± 2.71 to 19.1 ± 2.73 g DW · m^{-2} · d^{-1} at the different loading rates. At low light, production increased 2-fold when the loading rate of ADFDMW was increased from 2 to 4 L · d^{-1} ($P < 0.05$). In the 208-L system, these loading rates corresponded to initial concentrations of 2.2–4.5 mg · L^{-1} NH_4-N and 0.54–1.1 mg · L^{-1} SRP in the effluent (Table 1). Production rates leveled off at higher loading rates. Higher level of incident light (390 vs. 270 μmol photons · m^{-2} · s^{-1}) increased production significantly (Table 2). Relative content of ash-free DW (% DW) for algal biomass grown under all treatments was fairly constant, between 90% and 93%. Optimum biomass supported on the screen was about 80 g DW · m^{-2}. Physical limitations to production when

Table 1. Parameters of ATS effluent using different loading rates of anaerobically digested flushed dairy manure wastewater and incident light.

	Low light (270 μmol photons · m^{-2} · s^{-1})				High light (390 μmol photons · m^{-2} · s^{-1})		
Parameter	(n = 6)	(n = 6)	(n = 9)	(n = 5)	(n = 5)	(n = 5)	(n = 7)
ADFDMW input (L · d^{-1})	1.90 ± 0.15	3.67 ± 0.40	5.89 ± 0.33	8.80 ± 1.02	3.67 ± 0.47	5.80 ± 0.45	7.82 ± 0.92
NH_4-N (mg · L^{-1} · d^{-1})	2.13 ± 0.17	4.11 ± 0.45	6.60 ± 0.37	9.86 ± 1.14	4.11 ± 0.53	6.50 ± 0.50	8.76 ± 1.03
PO_4-P (mg · L^{-1} · d^{-1})	0.51 ± 0.04	0.98 ± 0.11	1.58 ± 0.09	2.36 ± 0.27	0.98 ± 0.13	1.56 ± 0.12	2.10 ± 0.25
TN (mg · L^{-1} · d^{-1})	3.77 ± 0.30	7.26 ± 0.80	11.67 ± 0.66	17.43 ± 2.02	7.26 ± 0.93	11.49 ± 0.89	15.49 ± 1.82
TP (mg · L^{-1} · d^{-1})	0.59 ± 0.05	1.14 ± 0.12	1.83 ± 0.10	2.73 ± 0.32	1.14 ± 0.15	1.80 ± 0.14	2.43 ± 0.29
ATS effluent							
NH_4-N (mg · L^{-1})	0.49 ± 0.25	0.73 ± 0.60	1.58 ± 0.73	1.23 ± 0.24	0.52 ± 0.36	1.57 ± 0.39	1.06 ± 0.15
NO_3-N (mg · L^{-1})	< 1	< 1	3.21 ± 2.73	3.84 ± 1.67	< 1	< 1	1.10 ± 1.65
TN (mg · L^{-1})	3.10 ± 0.93	4.70 ± 1.58	12.80 ± 4.43	14.79 ± 1.29	4.18 ± 0.50	11.06 ± 2.21	9.44 ± 1.62
PO_4-P (mg · L^{-1})	< 0.03	0.23 ± 0.28	0.95 ± 0.41	2.02 ± 0.11	0.04 ± 0.06	0.67 ± 0.32	1.36 ± 0.19
TP (mg · L^{-1})	0.22 ± 0.13	0.98 ± 0.52	2.58 ± 0.73	3.66 ± 0.43	0.45 ± 0.15	2.08 ± 0.45	2.35 ± 0.26

ADFDMW input is shown as volume added (L · d^{-1}), and daily increment to concentrations of NH_4-N, SRP, TN, and TP (mg · L^{-1} · d^{-1}) in effluent. The ATS effluent values are concentrations just before harvest. Values are means ± SD of measurements over five to nine harvest cycles. n, number of harvests.

TABLE 2. Means and mean comparisons of parameters for algal biomass and effluent in ATS grown under low and high light and different loading rates of anaerobically digested flushed dairy manure wastewater.

	Incident light		ADFDMW loading rate			
Factor	Low	High	$2\,L \cdot m^{-2} \cdot d^{-1}$	$4\,L \cdot m^{-2} \cdot d^{-1}$	$6\,L \cdot m^{-2} \cdot d^{-1}$	$9\,L \cdot m^{-2} \cdot d^{-1}$
Algal biomass						
Production ($g\,DW \cdot m^{-2} \cdot d^{-1}$)	14.8^{a}	17.2^{b}	7.60	14.7	15.6	17.7
N content (% w/w)	6.24	6.01	3.61	4.96^{a}	6.52^{b}	6.90^{c}
P content (% w/w)	1.08^{a}	1.01^{b}	0.63	0.88^{a}	1.18^{b}	1.06^{c}
N:P	12.8	13.0	12.9	11.9^{a}	12.3^{a}	14.4^{b}
C:N	8.19	7.85	12.5	8.98^{a}	7.61^{b}	7.46^{b}
C:P	110	114	174	125	98	112
TN-algae ($g \cdot m^{-2} \cdot d^{-1}$)	0.91	1.03	0.29	0.68^{a}	1.027^{b}	1.21^{b}
TP-algae ($g \cdot m^{-2} \cdot d^{-1}$)	0.16	0.17	0.05	0.13^{a}	0.18^{b}	0.19^{b}
TN-algae: TP-algae	5.85	5.88	6.14	5.40^{a}	5.68^{a}	6.51^{b}
Effluent						
NH_4-N ($mg \cdot L^{-1}$)	0.99	0.88	0.44	0.48^{a}	1.50^{b}	1.13^{b}
DIN:TN (%)	23.5^{a}	14.0^{b}	14.5	11.2^{a}	21.5^{b}	24.8^{b}
PO_4:TP (%)	39.8	32.1	<13	16.6^{a}	34.5^{a}	56.8^{b}
TN:TP (%)	6.55	5.15	16.8	8.29^{a}	5.18^{b}	4.07^{c}

The two-way ANOVA is shown for parameters that did not show significant interaction effects between light and loading rates. The treatment with 2-L loading rate, for which there was no high light treatment, was excluded from the 2-way ANOVA to combine the two light levels. The data shown for the 2-L loading rate represent means of replicates under low light treatment. Shared letters in the same row within a treatment (light or nutrient load) indicate no significant difference ($P > 0.05$). Nutrient content of algal biomass is shown as the weight of the nutrient relative to the dry weight of the biomass (% w/w). The ratio of nitrogen to phosphorus recovered in the algal biomass is shown as TN-algae:TP-algae.

the carrying capacity of the scrubber was exceeded included obstruction to flow and diffusion of water and nutrients, accumulation of particulates and debris on scrubber, self-shading of algae, and more turbid waters from algal fragments in effluent rendering less available light. Loss of particulates (mainly algal fragments in sieved water) while washing off screens led to underestimates in biomass and productivity values. Although variable under different growth rates, our estimate of biomass removed from pumps and collected from scrubber area after the screen was removed for harvest amounted to about 12% of total harvest.

Nutrients in algal biomass and effluent. Nitrogen and phosphorus content of algal biomass ranged from 2.9%–7.3% to 0.5%–1.3% (by weight), respectively. The N and P content increased significantly with loading rate, but P content reached a maximum at loading rate of $6\,L \cdot d^{-1}$, above which rate it showed a decrease (Table 2). At low light, a 3-fold increase in loading rate from 0.8 to 2.4 g TN (0.5–1.4 g NH_4-N) $\cdot m^{-2} \cdot d^{-1}$ increased the N content ($P < 0.05$) from 3.6% ± 0.57% to 6.6% ± 0.31%, after which it reached a plateau (Fig. 2a). Similarly at low light, an increase from 0.13 to 0.39 g TP (0.11–0.33 g SRP) $\cdot m^{-2} \cdot d^{-1}$ increased the P content ($P < 0.05$) from 0.63% ± 0.13% to 1.3% ± 0.14%, but it decreased at the highest loading rate (Fig. 2b). Algal biomass grown at the lowest loading rate appeared very light green in color and was light textured upon drying, compared with biomass grown at higher loading rates that had deep green color and relatively denser texture. Nutrient content was generally higher in algal biomass grown under relatively lower incident light. However, such variations were significant only

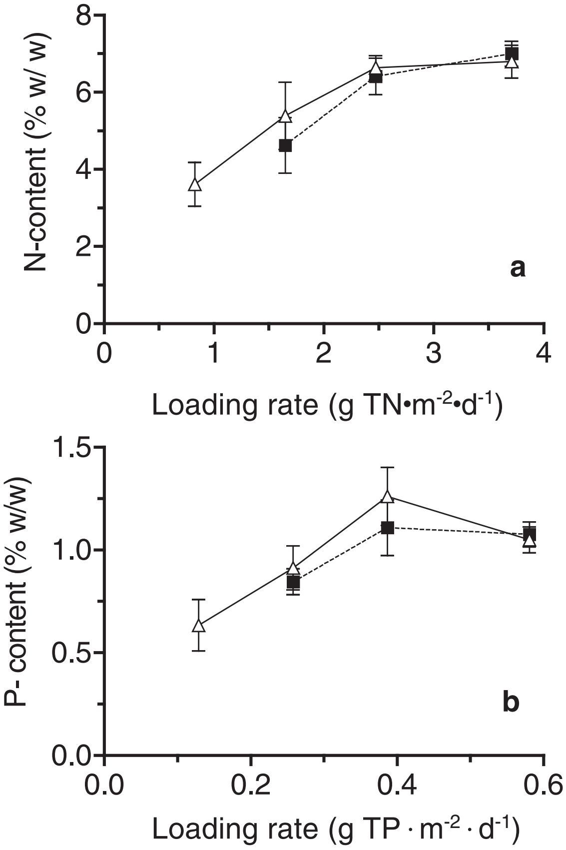

FIG. 2. Nitrogen (a) and phosphorus (b) content (%) of harvested algae under different loading rates of anaerobically digested flushed dairy manure wastewater and incident light. Data points represent mean values of five to nine measurements, and error bars are SDs. Symbols are as shown in Figure 1.

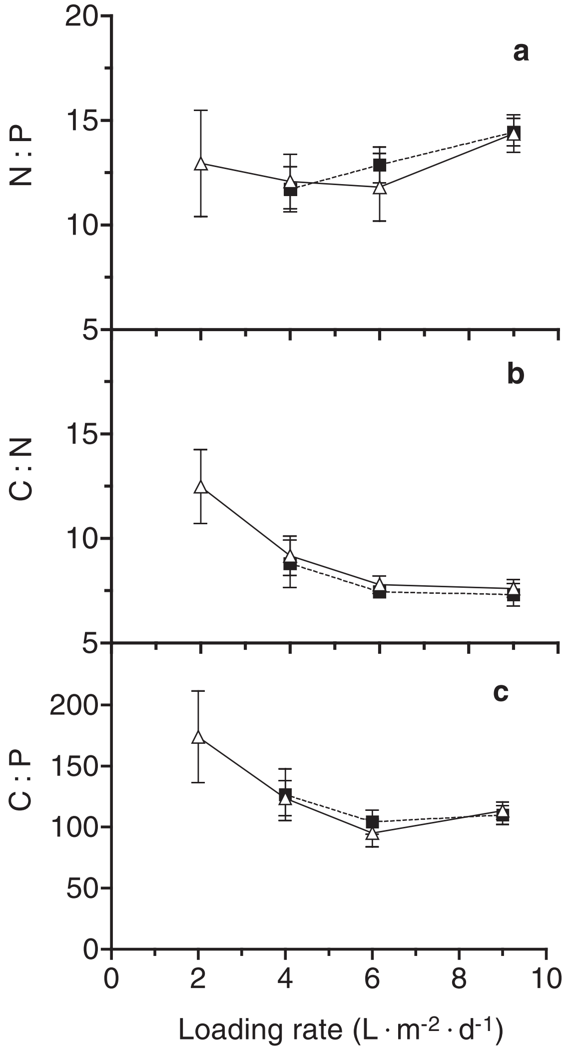

FIG. 3. Molar ratios of (a) N:P, (b) C:N, and (c) C:P in harvested algae under different loading rates of anaerobically digested flushed dairy manure wastewater and incident light (triangles, 270 μmol photons · $m^{-2} \cdot s^{-1}$; squares, 390 μmol photons · $m^{-2} \cdot s^{-1}$). Data points represent mean values of five to nine measurements, and error bars are SDs.

for P content (Table 2). The carbon content was fairly constant (42%–47%) with no significant difference ($P > 0.05$) at all loading rates and light regimes and an overall mean value of 45 ± 1.6.

Mean values for the N:P ratio (atomic) varied between 12 ± 1.1 at $4\,L \cdot d^{-1}$ to 14 ± 1.0 at $9\,L \cdot d^{-1}$ (Fig. 3a). The ratio was significantly higher at ADFDMW loading rate of $9\ L \cdot d^{-1}$, but the effect of light was not apparent (Table 2). The C:N ratio varied significantly ($P < 0.05$) from 12 ± 1.8 at the lowest to 7 ± 0.5 at the highest loading rate (Fig. 3b, Table 2). The C:P ratio varied between 95 ± 11.4 and 174 ± 37.5 (Fig. 3c) for all treatments, but differences were significant ($P < 0.05$) only with low light and an increase in loading rate from 2 to $4\,L \cdot d^{-1}$. Similar to C:N, the highest C:P ratios were observed at the lowest loading rate with low P content of algal biomass.

Average residual concentrations of dissolved inorganic nitrogen ($NH_4^+ + NO_3 + NO_2^-$-N) and phosphorus (SRP) in effluent at the end of a harvest cycle varied from about 12%–37% of TN and <13%–58% of TP, respectively (Table 1). Residual TN and TP in effluent increased with loading rate ($P < 0.05$). Under low light, average NO_3-N concentrations in effluent increased with loading rate from less than $1\,mg \cdot L^{-1}$ to a maximum of $3.8\,mg \cdot L^{-1}$ (26% of TN). Nitrate was markedly lower at high light, with concentrations of less than $1\,mg \cdot L^{-1}$ NO_3-N, except at the highest loading rate where it reached $1.1\,mg \cdot L^{-1}$ (12% of TN). Light showed a significant negative effect on the ratio of DIN:TN, but the effect was not evident on NH_4^+ levels (Table 2).

Nutrient removal efficiency, recovery, and mass balance. Removal efficiency of nutrients in effluent was complete (99%–100%) at all loading rates. Efficiency was calculated as

$$\text{Efficiency}\ (\%) = ((B + F) - A)/(B + F) \times 100\%$$

where B is the concentration of nutrient (N or P) in effluent at the beginning of a harvest cycle, F is the total concentration of nutrient input from ADFDMW over a cycle, and A is the residual concentration of nutrient in effluent at the end of a cycle (before harvest).

Recovery (%) of nutrients was calculated as output of TN and TP in algae (recovery in algae) or output in algae and effluent (total N or P recovery), divided by TN or TP in input, times 100% (Table 3). Nutrient recovery in algal biomass accounted for about 31%–52% of TN and 30%–59% of TP input. The recovery rates as TN and TP in algae ($g \cdot m^{-2} \cdot d^{-1}$) were significantly related to nutrient loading rate but not to incident light level (Table 2). The increase at the highest loading rate was not significant. Under low light, recovery rates for N increased with increasing loading rate ($P < 0.05$), with mean values ranging from 0.29 ± 0.15 ($n = 6$) $g\,N \cdot m^{-2} \cdot d^{-1}$ at a loading rate of $2\,L \cdot d^{-1}$ to 1.10 ± 0.15 ($n = 5$) $g\,N \cdot m^{-2} \cdot d^{-1}$ at $9\,L \cdot d^{-1}$. The increase for P recovery on the other hand was not significant ($P > 0.05$) above loading rate of $4\,L \cdot m^{-2} \cdot d^{-1}$. Algal production (Fig. 1) and nutrient content (Fig. 2) were coupled except for P content that decreased at the highest loading rate, where it appears to be uncoupled with the removal of N. The ratio of N to P recovered in algal biomass was 5.8 ± 1.0 ($n = 6$) at the loading rate of $4\,L \cdot m^{-2} \cdot d^{-1}$. With increases in loading rate from 4 to $9\,L \cdot m^{-2} \cdot d^{-1}$, the ratio varied from 5.5 ± 0.61 ($n = 6$) to 6.5 ± 0.41 ($n = 5$) at low light and from 5.3 ± 0.49 ($n = 5$) to 6.5 ± 0.32 ($n = 7$) at high light. The maximum removal rates of N and P per unit algal biomass ($mg \cdot g^{-1}\ DW \cdot m^{-2} \cdot d^{-1}$) were 70 and 13, respectively (Table 4).

Mass balance calculations (Table 3) showed that total recovery of nutrients in effluent and algal harvest was 50%–68% for N and 55%–73% for P. With increases in ADFDMW loading rate from 4 to $9\,L \cdot d^{-1}$, N and P recovery decreased at low light. In contrast, the

TABLE 3. Mass balance for ATS units grown under different loading rates of anaerobically digested flushed dairy manure wastewater and incident light.

Parameter	Low light (270 μmol photons · $m^{-2} \cdot s^{-1}$)				High light (390 μmol photons · $m^{-2} \cdot s^{-1}$)		
	($n = 6$)	($n = 6$)	($n = 9$)	($n = 5$)	($n = 5$)	($n = 5$)	($n = 7$)
Input (· $d^{-1} \cdot m^{-2}$)							
Volume of ADFDMW added (L)	1.90 ± 0.15	3.67 ± 0.40	5.89 ± 0.33	8.80 ± 1.02	3.68 ± 0.47	5.80 ± 0.45	7.82 ± 0.92
TN (g)	0.78 ± 0.06	1.47 ± 0.21	2.43 ± 0.14	3.63 ± 0.42	1.50 ± 0.19	2.39 ± 0.18	3.22 ± 0.38
TP (g)	0.12 ± 0.01	0.23 ± 0.03	0.38 ± 0.02	0.57 ± 0.07	0.23 ± 0.03	0.37 ± 0.03	0.50 ± 0.06
Output (· $d^{-1} \cdot m^{-2}$)							
Harvested algal biomass (g)	7.60 ± 2.71	15.0 ± 3.40	13.3 ± 4.32	16.2 ± 1.63	14.5 ± 3.67	18.0 ± 2.86	19.1 ± 2.73
TN in harvested algae (g)	0.29 ± 0.15	0.73 ± 0.16	0.91 ± 0.21	1.10 ± 0.15	0.63 ± 0.19	1.15 ± 0.15	1.31 ± 0.17
TN in ATS effluent (g)	0.13 ± 0.09	0.19 ± 0.13	0.38 ± 0.14	0.66 ± 0.21	0.14 ± 0.13	0.45 ± 0.11	0.82 ± 0.45
TP in harvested algae (g)	0.05 ± 0.01	0.13 ± 0.02	0.16 ± 0.03	0.17 ± 0.02	0.12 ± 0.03	0.20 ± 0.04	0.20 ± 0.03
TP in ATS effluent (g)	0.02 ± 0.02	0.04 ± 0.01	0.09 ± 0.03	0.14 ± 0.03	0.01 ± 0.02	0.07 ± 0.02	0.16 ± 0.07
TN-algae: TP-algae	6.14 ± 1.32	5.51 ± 0.62	5.55 ± 0.61	6.49 ± 0.41	5.29 ± 0.49	5.81 ± 0.39	6.52 ± 0.32
Recovery (%)							
N recovery in algae	38.4 ± 22.8	51.5 ± 14.1	37.5 ± 8.4	30.7 ± 5.0	42.0 ± 11.1	48.0 ± 5.3	41.1 ± 7.4
Total N recovery	60.9 ± 21.7	63.9 ± 10.6	53.2 ± 8.1	49.5 ± 10.1	50.5 ± 11.2	66.9 ± 8.0	68.2 ± 25.2
P recovery in algae	39.8 ± 14.4	59.4 ± 13.2	43.4 ± 9.9	30.3 ± 5.3	50.3 ± 9.9	53.2 ± 8.9	40.3 ± 7.1
Total P recovery	56.0 ± 15.9	72.8 ± 8.8	66.8 ± 10.2	55.0 ± 9.8	54.8 ± 12.8	73.3 ± 8.5	73.2 ± 24.4

Values are means ± SD of measurements over five to nine harvest cycles. *n*, number of harvests. The ratio of nitrogen to phosphorus recovered in the algal biomass is shown as TN-algae:TP-algae.

recovery at high light increased with increase in loading rate from 4 to 6 L · d^{-1} and reached a plateau at 9 L · d^{-1}. Two-way ANOVA showed a strong interaction between light and nutrient load (light × load) for total recovery of both N ($P = 0.0053$) and P ($P = 0.0076$). Loss of nutrients during harvest in unrecovered algal biomass and brown water may largely account for the disparity in mass balance (not quantified). Although grazer activity was minimized, the effect on biomass accumulation was not assessed and may partially contribute to the discrepancy.

The bulk of nutrient removed from wastewater is through bioassimilation by microbial flora and fauna of algal turfs. To a much lesser extent, nutrients can also be removed physically as organic and inorganic particulates trapped in turf biomass. Biomass collected from cleaning the scrubbers at harvest accounted for up to about 12% of total biomass and had an N content of 6.9%, about 30% higher than the corresponding harvested biomass (4.8%). Correction for this loss would raise recovery efficiency of N in harvested biomass by 5%–14%. Spatial heterogeneity occurred due to slight differences in water flow patterns, and temperature and irradiance caused variations in algal production and nutrient content within and among scrubbers, particularly under low light and low loading rates.

DISCUSSION

Comparing present with previous results from the scrubbers (Mulbry and Wilkie 2001, Wilkie and Mulbry 2002), algal production at a loading rate of 1 g TN · $m^{-2} \cdot d^{-1}$ increased from 5 to 9 g DW · $m^{-2} \cdot d^{-1}$. The increased algal production was due to increased irradiance level from an average of about 60 μmol photons · $m^{-2} \cdot s^{-1}$ (range, 40–140) in the previous

TABLE 4. Comparison of algal production and maximal nutrient removal rates in nutrient-rich domestic and agricultural effluents.

Experiment	Maximum algal production (g DW · $m^{-2} \cdot d^{-1}$)	Maximum removal rate (mg · $m^{-2} \cdot d^{-1}$)		Maximum removal rate per unit biomass (mg · g^{-1} DW algae)		N:P removal ratio	pH	References
		N	P	N	P			
Phormidium (batch culture)				78	146	0.5	> 8.5	Blier et al. 1996
Mixed periphyton	35[a]	1110	730	32	21	1.5	> 8.5	Craggs et al. 1996
Mixed periphyton	22[a]	1120[b]	157	52[c]	7[c]	7.3	6.4–7.6	Davis et al. 1990
Mixed periphyton	5	350	70	70	14	5.0	7.0–7.5	Mulbry and Wilkie 2001
Mixed periphyton	23	1330	210	70	13	5.4	7.0–7.5	This study

[a] Yearly mean values.

[b] Maximum value of 1990 at a different season.

[c] Calculated from values given in text.

experiments to about 270 μmol photons · $m^{-2} \cdot s^{-1}$ (range, 160–460) in the present study. The maximum algal production of 23 g DW · $m^{-2} \cdot d^{-1}$ falls well within values found for periphyton growth in similar systems (Davis et al. 1990, Adey et al. 1993, Craggs et al. 1996) treating agricultural runoff and effluent from wastewater treatment facilities (Table 3). The maximum productivity of 61 g DW · $m^{-2} \cdot d^{-1}$ reported in Craggs et al. (1996) far exceeds results compiled in Table 3, which the authors partly attribute to sedimentation, filtration, and precipitation of inorganic particulates, contributing to high ash content. The calculated value for the relative content of ash-free DW in Craggs et al. (1996) is only 45% of DW, which is about half of the values found for periphyton (80%–91% of DW) in previous (Wilkie and Mulbry 2002) and present studies in this laboratory and by Davis et al. (1990).

The N and P contents of algal biomass were distinctly lower than previous values measured for ATS grown at comparable loading rate of ADFDMW (1.05 g N and 0.12 g P · $m^{-2} \cdot d^{-1}$) but lower irradiance level (Mulbry and Wilkie 2001). The mean content in previous experiments was 7.1% ± 0.51% ($n = 9$) for N and 1.47% ± 0.05% ($n = 9$) for P, compared with this study where it was only about 3.6% for N and 0.65% for P. Low irradiance levels may promote higher production of photosynthetic pigments and higher N content in algal cells. Slower growth rates at relatively lower light may also allow for accumulation of nutrients. Ratios of N:P in harvested biomass in this study (mean, 12–14) are well within the range (5–19) given for nutrient-replete cultures of marine phytoplankton (Geider and La Roche 2002). Nutrient limitation studies on benthic algae by Hillebrand and Sommer (1999) showed that an N:P ratio less than 13 indicated N limitation and more than 22 indicated P limitation. Ratios of C:N exceeding 12 at the lowest loading rate reflect the low N content of algal biomass (3.6%), compared with the highest loading rate where the N content was about 7%. High ratios in this study depart from the Redfield ratio of about 6.6 (range, 6–9) considered typical for nutrient-replete cells containing about 45% protein (Geider and La Roche 2002). A relatively higher C:N ratio of around 7.5 is reported for benthic algae at the highest growth rates measured by Hillebrand and Sommer (1999). Although published records show large variations, high ratios of C:P in this study (83–245) exceed the range (27–135) given for marine phytoplankton grown under nutrient-replete conditions (Geider and La Roche 2002). Relatively higher N:P ratios and lack of increase in P removal rate at the highest loading rate applied suggest that algal potential for luxury consumption of P may have already been optimized. Sustained removal of N may be related to cyanobacteria that were relatively more prominent at higher loading rates. Cynobacteria can have higher N requirements for the light-harvesting phycobilisomes (Raven 1984) and can store inorganic nitrogen as cyanophycin granules (Simon 1973). Relatively higher C:N and C:P ratios in green algae with cellulose walls (Falkowski 2000) could partially explain the high ratios at low loading rates and the decrease at higher loading rates where filamentous cyanobacteria and diatoms were common turf algae. A shift in algal community structure toward cyanobacterial dominance was also found in outdoor raceways under higher summer temperatures and nutrient levels (Adey et al. 1993). Increased algal productivity with increasing loading rate was accompanied by decreases in C:N and C:P ratios. Because of the constrained and highly regulated nature of algal N and C metabolism, the C:N ratio may be used as an indicator of nutrient status (Clark 2001). High yields, low N content, and high C:N ratio at low loading rate indicate relatively higher carbohydrate accumulation, probably lipids, rather than protein synthesis. In marine phytoplankton, high values of C:N (> 12) are observed only when protein declines to about 25% of cell mass (Geider and La Roche 2002). In laboratory cultures of benthic algae, C:N and C:P ratios increased with decreasing growth rate, irrespective of the limiting nutrient in the case of the C:N ratio (Hillebrand and Sommer 1999).

Control of pH-mediated removal of nutrients contributed to relatively lower removal rates of N and P, compared with other studies where stripping of ammonia and precipitation of P at high pH accounted for a substantial portion of the removed N and P (Blier et al. 1996, Craggs et al. 1996). Maintaining the pH at 7–7.5 is a major difference from most other studies where increased pH due to algal photosynthesis (> 8.5) promotes volatilization of ammonia and precipitation of phosphorus with cations such as calcium and magnesium, as well as metals (Bender et al. 1994, Liehr et al. 1994), and results in algal biomass with a relatively low N:P ratio. Adey et al. (1993) measured mean P content of 0.34%–0.43% in harvested biomass of attached algal production on agricultural runoff, with relatively low P loading (0.012–0.148 mg · L^{-1} TP in source water). The high P content of harvested solids (mean, 2.1% of DW) from ATS systems running on domestic wastewater was considered to be due to precipitation of P, probably accounting for much of the removal by the ATS (Craggs et al. 1996). Comparison of similar studies on nutrient removal (Table 4) shows that production systems with pH < 7.6 result in similar and relatively high ratios of N:P (5–7) for nutrient removal per unit algal biomass, compared with systems with pH > 8.5 where the ratio is less than 2.

Loss of biomass in sieved water during harvest can be substantial as shown in Adey et al. (1993), where solids dried from brown water captured while harvesting by pressure sieve accounted for up to half of the total biomass. Applying a secondary sieve of smaller pore size to capture biomass passing through a larger sieve or using a vacuum for harvest would increase recovery of biomass and nutrients. Decreasing rates of nutrient recovery under low light and high ADFDMW loading rates may be related to the changes in algal turf composition that were prominent at the highest loading rate. This trend was not so apparent under high light.

Relatively smaller filaments of *Oscillatoria* spp. and diatoms, partially replacing the dominant large green algal filaments, would more likely pass through the 1-mm sieve used for harvesting. Although volatilization of ammonia is minimized at neutral pH, there may still be some loss in these nutrient-rich and turbulent systems. Bacterial denitrification in anaerobic microzones (such as the underside of the turf mat), as suggested in Baumgarten et al. (1999) and Craggs et al. (1996), may also partially account for the discrepancy in mass balance. However, total recovery efficiencies for N and P were similar, and a significant loss of N to the atmosphere through denitrification or volatilization would have resulted in a lower recovery efficiency for N than for P or a lower N:P ratio in the algal biomass.

In contrast to the high accumulation of nitrate in the previous study with the same ADFDMW (Mulbry and Wilkie 2001), nitrate levels were relatively insignificant in the present study even though much higher loading rates of N were applied. Temperature, pH, dissolved oxygen concentration, NH_4 availability, carbon quantity and quality, and C:N ratio are some of the factors considered to influence nitrification rates in aquatic systems (Strauss and Lamberti 2000). Considering the similar experimental setup for the two studies in this laboratory, it appears that the higher irradiance level enhancing algal growth was the overriding factor in controlling nitrification. Negligible nitrate concentrations and relatively lower ratios of DIN:TN in the effluent of ATS grown under high light support this observation. Bubbling with CO_2 in the present study may also have alleviated carbon limitation, likely to occur especially at the high loading rates where algal growth was enhanced.

Although more nutrients can be removed under high pH, algal production for treatment of nutrient-rich water under neutral pH minimizes stripping of ammonia to the atmosphere and yields utilizable biomass with balanced N:P ratios. Moreover, such systems can be coupled with processes that generate carbon dioxide as a byproduct such as anaerobic digestion. Integrating nutrient removal by algae with aquaculture is another promising application of phytoremediation, already shown with seaweed production in marine aquaculture (Chopin et al. 2001).

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