ELSEVIER

Bioresource Technology 84 (2002) 81–91

BIORESOURCE TECHNOLOGY

Recovery of dairy manure nutrients by benthic freshwater algae

Ann C. Wilkie [a,*], Walter W. Mulbry [b]

[a] *Soil and Water Science Department, University of Florida, P.O. Box 110960, Gainesville, FL 32611-0960, USA*
[b] *USDA/ARS, Soil Microbial Systems Laboratory, 10300 Baltimore Avenue, Beltsville, MD 20705, USA*

Received 5 March 2001; received in revised form 27 November 2001; accepted 4 December 2001

Abstract

Harnessing solar energy to grow algal biomass on wastewater nutrients could provide a holistic solution to nutrient management problems on dairy farms. The production of algae from a portion of manure nutrients to replace high-protein feed supplements which are often imported (along with considerable nutrients) onto the farm could potentially link consumption and supply of on-farm nutrients. The objective of this research was to assess the ability of benthic freshwater algae to recover nutrients from dairy manure and to evaluate nutrient uptake rates and dry matter/crude protein yields in comparison to a conventional cropping system. Benthic algae growth chambers were operated in semi-batch mode by continuously recycling wastewater and adding manure inputs daily. Using total nitrogen (TN) loading rates of 0.64–1.03 g m^{-2} d^{-1}, the dried algal yields were 5.3–5.5 g m^{-2} d^{-1}. The dried algae contained 1.5–2.1% P and 4.9–7.1% N. At a TN loading rate of 1.03 g m^{-2} d^{-1}, algal biomass contained 7.1% N compared to only 4.9% N at a TN loading rate of 0.64 g m^{-2} d^{-1}. In the best case, algal biomass had a crude protein content of 44%, compared to a typical corn silage protein content of 7%. At a dry matter yield of 5.5 g m^{-2} d^{-1}, this is equivalent to an annual N uptake rate of 1430 kg ha^{-1} yr^{-1}. Compared to a conventional corn/rye rotation, such benthic algae production rates would require 26% of the land area requirements for equivalent N uptake rates and 23% of the land area requirements on a P uptake basis. Combining conventional cropping systems with an algal treatment system could facilitate more efficient crop production and farm nutrient management, allowing dairy operations to be environmentally sustainable on fewer acres. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Benthic freshwater algae; Algal biomass; Nutrient recovery; Nitrogen; Phosphorus; Crude protein; Anaerobic digestion; Dairy manure; Sustainable agriculture; Proteinaceous biomass

1. Introduction

The vulnerability of ecosystems and groundwater to pollution has forced increasingly stringent environmental regulations to be imposed on dairy farms. However, harnessing solar energy to grow algal biomass on wastewater nutrients could provide a holistic solution to nutrient management problems at livestock operations. An algal treatment system concentrates nutrients into proteinaceous biomass by cultivating algae in engineered ponds or raceways, increasing the value and manageability of the nutrients. Harvested algal biomass is a high-grade protein source, which could be used to replace a portion of the protein content of animal feed imported onto the farm. Providing feed usually amounts to 50% or more of the cost of producing milk (Johnson et al., 1991). In addition, milk from dairy cows fed a diet supplemented with the marine alga *Schizochytrium* sp. showed an increase in omega-3-fatty acid content, a characteristic that has potential for improving consumer health (Franklin et al., 1999). The algal biomass could also be used as a slow-release fertilizer, either sprayed as a suspension directly on croplands or harvested and stored until conditions are favorable for spreading on the fields.

Previous investigators have cited the cost of drying the harvested algae as the biggest drawback to implementing the technology (Lincoln and Earle, 1990). However, when considered in conjunction with an anaerobic digestion system where energy is recovered from manure, the cost of drying the harvested algae could be minimal (Wilkie, 2000a). Also, since anaerobic digestion increases the availability of manure nutrients, the combination of anaerobic digestion followed by algal production could be synergistic. As well as producing energy in the form of methane gas, anaerobic digestion

---

[*] Corresponding author. Tel.: +1-352-392-8699; fax: +1-352-392-7008.
E-mail address: acwilkie@mail.ifas.ufl.edu (A.C. Wilkie).

PII: S0960-8524(02)00003-2

lowers the odor-producing potential of a wastestream by degrading malodorous volatile compounds and reduces the levels of pathogenic organisms by exposing them to a detrimental environment (Wilkie, 2000a,b). Nutrients, such as N and P, contained in the organic compounds are conserved and mineralized to more soluble and biologically available forms.

Several possibilities exist for replacing conventional cropping for nutrient recovery with aquatic-based cropping systems, including the use of wetlands (Tanner et al., 1995), water hyacinths (Costa et al., 2000), duckweed (Bonomo et al., 1997), high-rate algal ponds (Fallowfield et al., 1992), and cyanobacteria (Crawford et al., 1993; Lincoln et al., 1993). There is a considerable literature on the treatment of municipal wastewater in high-rate ponds (Nurdogan and Oswald, 1995) and on the treatment of dairy and swine manures by monoalgal cultures such as *Spirulina* (Lincoln and Wilkie, 1995; Lincoln et al., 1996; Olguin et al., 1997).

Benthic (attached) freshwater algae may have an advantage over planktonic (suspended) algae in the ease of separation and recovery of algal biomass from an aqueous stream. Many species of benthic freshwater algae are only found in lotic environments (high current velocity zones) and fail to grow when placed in lentic (still-water) environments (Whitford and Schumacher, 1961). This unique aspect of their physiological requirements may give benthic algae an additional advantage over planktonic algae in nutrient recovery applications. Whitford (1960) has demonstrated that a shear velocity of at least 15 cm s^{-1} is required to produce a steep diffusion gradient, which places bulk fluid nutrient concentrations close to algal surfaces and enhances nutrient uptake. In experiments with *Oedogonium*, Whitford and Schumacher (1961) demonstrated a 10-fold increase in P uptake rates and higher respiration rates at a shear velocity of 18 cm s^{-1} compared to that in still-water. While planktonic algae deplete surrounding nutrients and become diffusion-limited in their nutrient-uptake rates, benthic algae exposed to high shear velocities are not diffusion-limited and may achieve higher uptake rates at similar concentrations and light levels.

Technology for cultivating benthic algae was perhaps first documented by Skadovski (1961) and developed from observations of periphyton in waterworks. Later, Barna and Nagy-Toth (1980) exploited the surface area of benthic algae to reduce the temperature of thermally polluted waters by growing a mixed culture of *Ulothrix*, *Stigeoclonium* and *Cladaphora* on horizontal plastic screens. Expanding on Skadovski's work, Sladeckova et al. (1983) and Vymazal (1988) further developed the "bioeliminator" benthic algae system for pre-treating river water prior to potable water treatment. They experimented with several different systems in which plastic mesh screens were placed, either horizontally or vertically, in the flow stream and found that, in their continuous system, species of *Stigeoclonium* and *Oedogonium* were dominant. Besides plastic mesh screen, horizontal open-cell styrofoam has also been used for benthic algae cultivation (Bothwell, 1983), as well as rotating styrofoam disks (Przytocka-Jusiak et al., 1984). Wood (1987) developed an effluent selector designed to reduce washout of *Stigeoclonium* that was cultured as suspended spherical clusters.

Commercial application of benthic algae cultivation technology in the US began with Adey and coworkers, who developed the "algal turf scrubber" (ATS) to purify marine waters for microcosm research on shallow reefs (Adey and Hackney, 1989). The essential elements of the ATS system are a solid support for the growth and harvest of benthic algae, wave surge, and maximal light. A frequent wave surge created by a tipping bucket prevents development of boundary layers that limit nutrient and metabolite exchange, as well as preventing light shielding of interior portions of the algae (Adey and Loveland, 1998). Small-scale algal growth chambers are used in aquaria, in a variety of mesocosms, and for the commercial production of coral reef organisms (Adey and Loveland, 1998). Large-scale ATS units are in commercial use for treating wastewater generated by tilapia aquaculture operations (Adey and Loveland, 1998), agricultural runoff (Adey et al., 1993), and for N and P removal in tertiary treatment of municipal wastewater (Craggs et al., 1996a,b).

A recent development using benthic algae technology is proposed by Drenner et al. (1997) and further characterized by Rectenwald and Drenner (2000). In this "ecological water treatment system", the benthic algae are grown on vertical plastic screens and are harvested by grazing fish. A conical tank bottom collects fish feces. N and P are recovered by harvesting both fish and feces. Experiments on municipal wastewater effluent resulted in a 23% reduction in N and a 82% reduction in P (Rectenwald and Drenner, 2000).

None of the benthic algae technologies have been applied to nutrient recovery from dairy manure streams and there are no studies on the nutrient content of the harvested algae toward a goal of using it as a feed or fertilizer product. The purpose of this study was to assess the ability of benthic freshwater algae to recover nutrients from dairy manure and to evaluate nutrient uptake rates and dry matter/crude protein yields in comparison to a conventional cropping system.

2. Methods

2.1. Collection and characterization of dairy manure

Three dairy manure sources were employed in the benthic algae growth studies. Two of the manures used were collected from the Dairy Research Unit (DRU) of the USDA/ARS facility in Beltsville, MD (USDA) and stored at 4 °C prior to use. The USDA dairy houses 225 mature cows and 225 replacement heifers in a confinement operation where cows are bedded on rubber pads with supplemental sawdust added twice weekly. The manure is mechanically scraped from the barn at around 12% total solids (TS) and fed to a screw-press separator where recovered solids are removed for use as a soil amendment. The filtrate (5.3% TS) is fed to an unmixed 380 m^3 cylindrical concrete anaerobic digester heated to 35 °C and operated at a 23-day hydraulic retention time (HRT) (Wilkie et al., 1995). This filtrate comprised the USDA undigested manure and the anaerobic digester effluent (3.2% TS) comprised the USDA digested manure used to cultivate benthic algae in this study.

The third source of dairy manure was anaerobically digested manure collected from the DRU of the University of Florida in Gainesville, FL (UF), which was shipped overnight to Beltsville, MD, and stored at 4 °C prior to use. The UF DRU houses a 500-cow milking herd, bedded on sand, with manure removed by freshwater flushing (Wilkie, 2000b). Flushed dairy manure is conveyed across a sand trap (where sand is recovered for bedding) to a mechanical separator which removes large fibrous materials for direct land application. The remaining liquids pass through a gravity settling basin prior to entering a digester influent sump from where they are pumped to pilot-scale fixed-film anaerobic digesters at 0.4% TS. The UF digested dairy manure used for benthic algae growth was a composite sample collected from the effluent of four 340 L fixed-film digesters. All four digesters were operated at a 3-day HRT at 35 °C. The TS of the effluent averaged 0.27%.

The manures from the USDA and UF facilities differed significantly (Table 1) because the USDA DRU uses a mechanical scrape system for cleaning the barns, while the UF DRU uses a hydraulic flushing system. The USDA and UF dairies also use different types of animal bedding (sawdust and sand, respectively). In both systems, the manures are subjected to a solids separation step followed by anaerobic digestion of the separated liquids. Since no water is used for flushing, the USDA digested manure has a 10 to 11-fold higher content of total nitrogen (TN) and total phosphorus (TP) compared to the UF digested manure. However, the TN:TP ratios of both digested manures are similar (ca 9–10). The USDA undigested manure has a much lower TN:TP ratio of 4 compared to that of USDA digested manure, presumably due to precipitation of P with solids during anaerobic digestion and unusually high levels of TN in the USDA digested manure samples

Table 1
Characteristics of dairy manures

Characteristics	Manure source		
	USDA undigested	USDA digested	UF digested
Manure management	Scraped and separated	Scraped, separated and anaerobically digested	Flushed, separated and anaerobically digested
Type of anaerobic digester	–	Full-scale suspended growth	Pilot-scale fixed-film
Digester retention time (d)	–	23	3
Digester operating temperature (°C)	–	35	35
TS (mg L^{-1})	53200	31600	2670
	(±116)[a]	(±195)	(±40)
VS (mg L^{-1})	40300	20900	1310
	(±84)	(±154)	(±5)
SS (mg L^{-1})	39300	23700	888
	(±1430)	(±579)	(±11)
COD_t (mg L^{-1})	71800	32700	1610
	(±1240)	(±1990)	(±29)
COD_s (mg L^{-1})	19300	4900	376
	(±913)	(±45)	(±9)
TN (mg L^{-1})	1210	2370	225
	(±194)	(±123)	(±15)
TKN (mg L^{-1})	1210	2370	225
	(±194)	(±123)	(±15)
NH_4–N (mg L^{-1})	306	1620	178
	(±49)	(±341)	(±13)
NO_3–N (mg L^{-1})	< 1	< 1	< 1
	nd	nd	nd
TP (mg L^{-1})	303	240	24.7
	(±55)	nd	(±3)
COD:N:P ratio	237:4:1	136:10:1	65:9:1
pH	6.95	7.83	7.64
Conductivity (mS cm^{-1})	15.60	16.20	3.11

[a] Values in parentheses represent standard deviations of triplicate subsamples.

used in this study. Normally, TN levels in digested manure should be similar to those of the undigested manure. In addition, the ammonium nitrogen (NH_4–N) content of the USDA digested manure was 5-fold higher than that of the USDA undigested manure due to the mineralization of organic N during digestion.

2.2. Benthic algae growth chambers

Two benthic algae growth chambers (BAGCs), each containing 0.93 m^2 of growing area, were obtained from Walter Adey (Smithsonian Institution, Washington, DC). The BAGCs were operated in semi-batch mode by continuously recycling 220 L of wastewater contained in plastic drums across a tipping bucket which provided wave action to reduce diffusional resistance (Fig. 1). The BAGCs were seeded with algal consortia using water from a nearby stream (Beltsville, MD). The benthic algae were initially established on square screens (96.5 cm sides) of black polyethylene (3 × 4 mm mesh; InterNet, Minneapolis, MN) at ambient laboratory temperature (22 °C) using 175 W metal halide lights, a 16 h photo period, a flow rate of 110 L min^{-1}, and daily additions of USDA digested manure (at a loading of approximately 0.25 g NH_4–N m^{-2} d^{-1}). Poor growth of benthic algae was overcome by replacing 75 L of BAGC wastewater with deionized water once a week. Photosynthetic photon flux density on the screens ranged from 40 to 140 μmol s^{-1} m^{-2}, with the maximum intensity in the center of the chamber. To minimize ammonia emissions, the pH of the liquid phase was maintained at pH 7.0–7.5 by periodic addition of hydrochloric acid as required to counter for alkalinity loss due to photosynthesis.

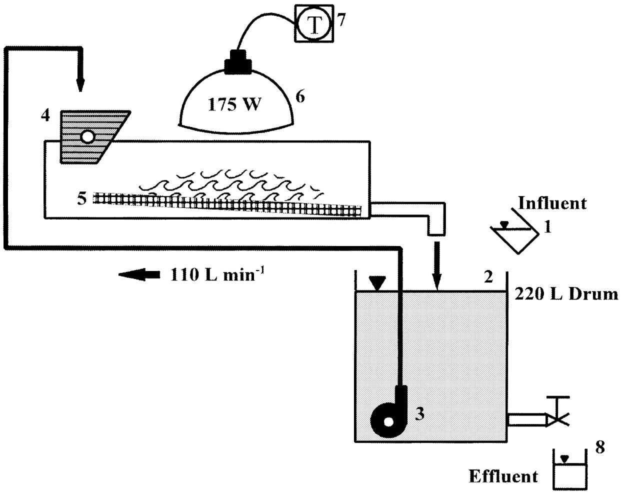

Fig. 1. Schematic of benthic algae growth chamber (BAGC) setup. Components are: (1) daily influent addition (manual); (2) plastic drum (220 L active volume); (3) submersible recirculation pump (110 L min^{-1}); (4) tipping bucket; (5) black polyethylene screen to support algal growth (96.5 cm × 96.5 cm screen with 3 × 4 mm mesh, 0.93 m^2); (6) 175 W metal halide lamp; (7) timer set at 16 h photo period; (8) weekly effluent collection (manual).

After the algae were established, daily additions of manure were progressively increased each week until the amounts reached 0.64–1.03 g TN m^{-2} d^{-1}. Algal biomass was harvested weekly by removing the screens to a flat surface and scraping off the biomass using a rigid plastic ruler. Screens were then returned to the units and 75 L of the BAGC wastewater was replaced with deionized water. After a quasi-steady state was achieved (based on algal yields), the BAGCs were operated for 9-week periods on one of the dairy manures, with the USDA undigested manure feeding period following the 9-week feeding period for the USDA digested manure (on the same BAGC). Typically, 0.29 L of USDA digested and 0.49 L of USDA undigested manure were added daily to the BAGC unit. The second BAGC received 4.26 L of UF digested manure daily.

2.3. Chemical analyzes

Harvested algal biomass was dried at 60 °C prior to analysis for total Kjeldahl nitrogen (TKN), TP, and elemental composition. BAGC effluent samples (Fig. 1) were collected and stored at 4 °C prior to analysis for TKN, TP, NH_4–N, nitrate (NO_3–N), conductivity (EC), and total chemical oxygen demand (COD_t).

Measurements of total solids (TS), suspended solids (SS), volatile solids (VS), COD_t, soluble COD (COD_s), pH, and EC were performed according to standard methods (APHA, 1995). Measurements of TKN, TP, NH_4–N, NO_3–N, and elemental analyses were performed according to USEPA methods (USEPA, 1983). TN was determined as the sum of TKN plus NO_3–N, and organic N was determined as the difference between TKN and NH_4–N. Crude protein was calculated as 6.25 times TKN.

2.4. Statistical methods

Data from weekly samples for each run were treated as nine replicates from a sample population. The significance of differences between sample means was tested using the Student's t-test for independent samples and unequal variances (Steel and Torrie, 1980). The means and sample standard deviations were entered into a Microsoft Excel 97 spreadsheet, the appropriate variance and effective degrees of freedom were determined, and the results were subjected to a two-tailed t-test at a confidence level of $\alpha = 0.01$, unless stated otherwise.

3. Results

3.1. Algal species in the BAGC

Algal species growing on the BAGC screens at the conclusion of these experiments were identified by microscopy as *Microspora willeana* Lagerh (the most abundant alga present on the screens), *Ulothrix zonata* (Weber & Mohr) Kütz, *Ulothrix aequalis* Kütz, *Rhizoclonium hieroglyphicum* (C.A. Agardh) Kütz, and *Oedogonium* sp.

3.2. Algal yields and N and P uptake

The average daily algal production rates (dry matter basis) for the 9-week periods on the three dairy manures were not statistically different (Table 2), despite differences in TN, NH_4–N, TP and COD loading rates. The dried algal production rates were typically 5 g m^{-2} d^{-1} regardless of the manure source or loading rate. The algae TN uptake rates for both the digested and undigested USDA manure were not significantly different ($\alpha = 0.01$) at 0.26 and 0.27 g TN m^{-2} d^{-1} and this follows logically from the corresponding TN loading rates for the two USDA manures, which were also not statistically different. In contrast, the algae grown on the UF digested manure exhibited a significantly higher TN uptake rate of 0.39 g m^{-2} d^{-1}, and this corresponds with a significantly higher TN loading rate imposed during the 9-week feeding period.

The TP uptake rates for algae grown on the three manures were significantly different only at an $\alpha = 0.1$ level, in spite of significantly different TP loading rates imposed during the 9-week trial periods (Table 2). The small differences in TP uptake rates as well as the relatively high portion of applied P which was recovered in the algae suggest that P may have been limiting. Only the algae grown on undigested USDA manure exhibited a TP uptake rate which was significantly ($\alpha = 0.01$) lower than the applied TP loading rate, indicating that in this case excess P may have been available. The N:P ratio (Table 1) for the undigested USDA manure was low (4:1) compared to the digested manures (9:1 and 10:1), and this could account for the difference between P loading and uptake rates. TP measurements of the algae included organic P as well as P which may have precipitated within the algal biomass; therefore, physicochemical differences between the diluted manures rather than algal uptake could be responsible for these differences.

3.3. Analysis of algal biomass

For each week during the 9-week periods, harvested algal biomass was dried and analyzed. The yield, crude protein and elemental analysis of the algae grown from the three manure sources are compared in Table 3 to literature values for corn silage (a typical dairy feed ration ingredient) and other benthic freshwater algae grown on secondary municipal wastewater. While the N content for algae grown on both the digested and undigested USDA manure was similar (4.9%), the level of N in the algae grown on UF digested manure was substantially higher (7.1%), possibly due to the higher N

Table 2
Daily average N, P and COD loading rates, algal production rates and nutrient uptake rates over the 9-week feeding period for each dairy manure

	Manure source		
	USDA undigested	USDA digested	UF digested
Average daily addition (L)	0.49 a (±.043)[a]	0.29 b (±.060)	4.26 c (±1.460)
Average loading rates			
TN (g m^{-2} d^{-1})	0.64 a (±.056)	0.75 a (±.154)	1.03 b (±.353)
NH_4–N (g m^{-2} d^{-1})	0.16 a (±.014)	0.51 b (±.105)	0.81 c (±.280)
TP (g m^{-2} d^{-1})	0.16 a (±.014)	0.08 b (±.016)	0.11 c (±.039)
COD_t (g m^{-2} d^{-1})	37.9 a (±3.31)	10.3 b (±2.12)	7.35 c (±2.52)
COD_s (g m^{-2} d^{-1})	10.2 a (±.888)	1.55 b (±.318)	1.72 b (±.591)
Average algal production rates			
Dry algae (g m^{-2} d^{-1})	5.35 a (±1.52)	5.34 a (±2.20)	5.53 a (±1.30)
Average nutrient uptake rates			
TN (g m^{-2} d^{-1})	0.27 a (±.076)	0.26 a (±.108)	0.39 b (±.092)
TP (g m^{-2} d^{-1})	0.11 a (±.031)	0.08 b (±.033)	0.08 b (±.019)

[a] Values in parentheses are standard deviations ($n = 9$). Values in the same row with the same letter are not statistically different at an $\alpha = 0.01$ level using a Student's *t*-test for independent samples and unequal variances (except for TP uptake rates, where $\alpha = 0.1$).

Table 3
Yield, crude protein, and elemental composition of dried algal biomass compared to corn silage and other benthic algae

	Manure source			Corn silage[a]	Other benthic algae[b]
	USDA undigested	USDA digested	UF digested		
DM yield (kg ha^{-1} yr^{-1})	19500	19500	20200	15700	72000
Crude protein (% of DM)[c]	31	31	44	7	19
N (mg kg^{-1})	49900	49200	70900	11000	30900
	(±6340)[d]	(±8680)	(±4400)	nd[e]	nd
P (mg kg^{-1})	20600	15400	14700	2500	20720
	(±5030)	(±1710)	(±2240)	nd	nd
K (mg kg^{-1})	16300	11300	17200	10900	7230
	(±3120)	(±1350)	(±1580)	nd	nd
Ca (mg kg^{-1})	10900	8040	18400	3600	33700
	(±2130)	(±1720)	(±3760)	nd	nd
Mg (mg kg^{-1})	5340	4520	4170	1800	29500
	(±1290)	(±532)	(±448)	nd	nd
B (mg kg^{-1})	20.3	21.0	26.0	nd	nd
	(±4.6)	(±5.8)	(±2.0)		
Cu (mg kg^{-1})	84.1	55.9	106.0	5.0	30
	(±17.6)	(±24.0)	(±13.3)	nd	nd
Fe (mg kg^{-1})	1790	1060	754	nd	14800
	(±454)	(±262)	(±230)		nd
Mn (mg kg^{-1})	310	241	304	70	320
	(±72)	(±38)	(±42)	nd	nd
Mo (mg kg^{-1})	2.74	2.28	1.38	nd	nd
	(±0.88)	(±0.22)	(±0.20)		
Si (mg kg^{-1})	158	100	1640	nd	nd
	(±54.9)	(±18.8)	(±370)		
Zn (mg kg^{-1})	396	223	419	33	170
	(±72.0)	(±70.0)	(±81.0)	nd	
Al (mg kg^{-1})	802	583	2170	nd	17060
	(±184)	(±93.3)	(±612)		nd
Ba (mg kg^{-1})	29.8	24.6	11.1	nd	nd
	(±11.20)	(±8.99)	(±7.89)		
Cd (mg kg^{-1})	0.41	0.41	0.18	nd	nd
	(±0.08)	(±0.13)	(±0.10)		
Ni (mg kg^{-1})	3.8	2.0	2.2	nd	nd
	(±0.78)	(±0.65)	(±0.86)		
Pb (mg kg^{-1})	5.9	7.7	4.5	nd	nd
	(±1.4)	(±2.4)	(±1.3)		

[a] Corn silage data from NRCS (1999).
[b] Other benthic algae data from Craggs et al. (1996a,b).
[c] Crude protein calculated as 6.25 times TKN content on dry matter (DM) basis.
[d] Values in parentheses are standard deviations ($n = 9$).
[e] nd – no data given.

loading applied during the UF digested manure feeding period.

When converted to crude protein, these levels indicate a dry matter protein content of 31% for algae grown on both digested and undigested USDA manure and 44% for the algae from UF digested manure, compared to a typical corn silage protein content of 7%. While the annual dry matter yields are only slightly higher than that for corn silage, the higher protein contents suggest a potential for algae to produce higher yields of protein with correspondingly higher levels of N uptake. The data shown for other benthic algae (Craggs et al., 1996a,b) indicates an almost 4-fold higher dry matter yield is possible (Table 3). However, while the ash contents of algae grown on USDA undigested and digested, and UF digested manures were only 12.1%, 11.0% and 13.6% (data not shown), respectively, the other benthic algae had an ash content of 54.8% (Craggs et al., 1996b). This accounts for the lower protein level found in the other benthic algae (19%). Yet, this protein level is still higher than that for corn silage.

Although the N levels of the undigested and digested USDA manure-grown algae were similar, the P level of the undigested manure-grown algae was higher than that of the digested manure-grown algae, 2.1% and 1.5%, respectively (Table 3). The higher P levels of the undigested USDA manure-grown algae were likely due to the lower N:P ratio of this manure (Table 1). The P levels of the algae grown on the USDA digested and UF digested manures were similar.

Algae grown using the three manures were roughly similar with respect to Mg, Mn, B, and Ni. However, algae grown using digested UF manure were approximately twice as high with respect to Ca, K, Zn, Cu, and Al content and approximately one half as high with respect to Fe, Cd, Pb, Ba, and Mo content compared to algae grown on digested USDA manure. Silica levels of the UF digested manure-grown algae were approximately 10-fold higher than levels in the USDA manure-grown algae. This is probably due to the use of sand as bedding material at the UF dairy. Algae grown on undigested USDA manure had higher levels of nearly all metals compared to algae grown on USDA digested manure.

3.4. N and P removal from manure by BAGC

Mean levels of $\mathrm{COD_t}$, TN, TP, $\mathrm{NO_3}$–N and EC in the BAGC effluents at weekly algae harvest are shown in Table 4, along with estimates for steady-state concentrations assuming zero uptake and the corresponding treatment efficiencies. $\mathrm{COD_t}$ values of BAGC effluents were significantly lower than steady-state estimates for all three manures. The highest COD reduction (95%) resulted from algae grown on undigested USDA manure, which contained the highest level of COD. COD loading of BAGC units was approximately 37.9 g $\mathrm{m^{-2}\,d^{-1}}$ using the USDA undigested manure, 10.3 g $\mathrm{m^{-2}\,d^{-1}}$ using USDA digested manure and 7.4 g $\mathrm{m^{-2}\,d^{-1}}$ for the UF digested manure (Table 2). In spite of having a lower COD loading rate, the effluent from the UF digested manure-fed BAGC contained COD levels similar to levels in the BAGC fed USDA undigested manure, and exhibited the lowest COD treatment efficiency (77%). The UF digested manure had the lowest portion of soluble COD relative to the total COD (Table 1), indicating a high level of stabilization for this manure such that the remaining COD would likely be poorly degradable and remain in the effluent after treatment. This accounts for the lower levels of COD removal using the UF digested manure.

The effluent in all BAGC runs contained very low levels of $\mathrm{NH_4}$–N (< 1 mg $\mathrm{L^{-1}}$, data not shown), as the $\mathrm{NH_4}$–N fed was either assimilated by the algae, lost to $\mathrm{NH_3}$–N volatilization, or nitrified to $\mathrm{NO_3}$–N. High nitrification rates led to significant nitrate levels in BAGC effluents (Table 4). With all three manures, production of $\mathrm{NO_3}$ accounted for a significant portion of residual N in the effluent. Nitrate levels were lowest with undigested manure, suggesting that lower inputs of $\mathrm{NH_4}$–N, higher levels of soluble carbon and/or other components in undigested manure might inhibit $\mathrm{NO_3}$ production.

Levels of P measured in the BAGC effluents did not correspond with the P loading rates. While P uptake rates in Table 2 suggest that P was limiting in digested manure, the effluent data in Table 4 indicates that higher levels of P remained in these effluents in conflict to the

Table 4
BAGC effluent parameters and treatment efficiencies for dairy manures

BAGC effluent parameters	Manure source		
	USDA undigested	USDA digested	UF digested
Steady-state estimates (assuming zero uptake)[a]			
$\mathrm{COD_t}$ (mg $\mathrm{L^{-1}}$)	3300	895	639
TN (mg $\mathrm{L^{-1}}$)	64	78	128
TP (mg $\mathrm{L^{-1}}$)	14	7	10
EC (mS $\mathrm{cm^{-1}}$)	0.72	0.44	1.24
Actual measurements[b]			
$\mathrm{COD_t}$ (mg $\mathrm{L^{-1}}$)	169	92.3	148
	(±44.2)[c]	(±12.0)	(±43.0)
TN (mg $\mathrm{L^{-1}}$)	25.7	29.5	77.5
	(±13.2)	(±5.49)	(±23.6)
TP (mg $\mathrm{L^{-1}}$)	1.02	1.97	4.86
	(±.32)	(±1.21)	(±2.03)
$\mathrm{NO_3}$–N (mg $\mathrm{L^{-1}}$)	10.5	19.0	63.0
	(±8.02)	(±3.53)	(±9.69)
EC (mS $\mathrm{cm^{-1}}$)	0.8	0.5	1.2
	(±.12)	(±.02)	(±.19)
Treatment efficiencies[d]			
COD reduction (%)	95	90	77
TN reduction (%)	60	62	39
TP reduction (%)	93	70	51

[a] Steady-state concentrations were calculated by multiplying the parameter concentration by the average volume added per week, dividing by the 75 L weekly dilution and, for TN, adjusting for nitrate concentration.

[b] Samples for parameter measurements were taken just prior to weekly algae harvest.

[c] Values in parentheses are standard deviations ($n = 9$).

[d] Treatment efficiencies are based on reduction of measured values to that of steady-state estimates.

uptake data. It is likely that measurement errors of effluent P levels near the 1 mg L^{-1} detection limit of our assays, magnified by factors accounting for the high volume of the BAGC reservoirs, contributed to these errant results. It is also likely that occasional measurement errors may have been introduced because of accidentally sampling prior to refilling the BAGC reservoir to compensate for evaporative water loss. The P uptake data derived from harvested algae analysis are not subject to these same errors of measurement.

Conductivity values in BAGC effluents varied considerably depending on the manure source, ranging from 0.5 to 1.2 mS cm^{-1} using digested USDA and digested UF manure, respectively. In all cases, the measured EC in the BAGC effluents was close to the values predicted by the steady-state estimates (Table 4).

4. Discussion

4.1. Benthic algae as feed

There are few studies on the use of benthic freshwater algae as feed, and only a few reports were found which considered any of the five species identified in the present study. Recognizing the potential of benthic freshwater algae as a feed, Hindak and Pribil (1968) characterized several species after results of growth experiments indicated both higher biomass and protein yields of benthic species compared to the reference planktonic species. While species of benthic alga, *Ulothrix* and *Stigeoclonium*, had slightly lower levels of crude protein (45% and 52%, respectively) than the planktonic reference strain of *Scenedesmus* (54% crude protein), their yields were 50 and 43 g m^{-2} d^{-1}, respectively, compared to only 33.4 g m^{-2} d^{-1} for *Scenedesmus*. All essential amino acids were found in the benthic species. Even more promising were the results of protein digestibility studies which indicated that, without pretreatment, *Ulothrix* and *Stigeoclonium* exhibited in vitro digestibility of 91% and 90%, respectively, compared to only 71% for *Scenedesmus*. Hindak and Pribil (1968) theorized that the lamellar structure of the benthic algal cell walls enhanced entry of peptic enzymes and was responsible for higher protein availability.

Ionescu (1970) observed *Ulothrix* with a maximum growth of 15.6 g m^{-2} d^{-1} in summer months in a saline lake, but was only able to achieve a laboratory growth rate of 3.6 g m^{-2} d^{-1}. However, the natural *Ulothrix* had a protein content of 29.9% and was 30% ash, whereas the laboratory culture supplemented with $(NH_4)_2HPO_4$ had a protein content of 58.8% and was only 22% ash. Boyd (1973) examined the protein content of several planktonic and benthic freshwater algae sampled from natural environments and found crude protein levels of 19.75% and 16.5% for species of *Rhizoclonium* and *Oedogonium*, respectively. Fekete et al. (1972) successively harvested *Rhizoclonium* from a pond and obtained a maximum yield of 5.9 g m^{-2} d^{-1} and a 25% crude protein level. An algal powder made from *Oedogonium obtruncatum* had a crude protein content of 29.8% (Singh, 1970) and was used successfully as the principal protein source in the diet of carp (*Cirrhinus mrigala*). Similarly, an algal powder made from *Cladophora glomerata* (31% crude protein) constituted over 50% of the protein in feed for tilapia (*Sarotherodon niloticus*) which yielded acceptable food conversion ratios (Appler and Jauncey, 1983). By comparison, crude protein levels for algae grown on dairy manures in this study were 31% (undigested and digested USDA manure) and 44% (UF digested manure).

Human consumption of benthic freshwater algae is not well-documented but, according to Johnston (1970), a species of *Oedogonium* was sold in markets in India. A study of the fatty acid composition of *Ulothrix aequalis* lipids showed that more than 70% of the lipid fraction in this benthic freshwater algae was comprised of omega-3 fatty acids, which indicates a potential for health benefits from consuming some freshwater algal species (Jamieson and Reid, 1976). Supplementation of poultry feed with 1% of lipids extracted from *Nannochloropsis* sp. (Eustimatophyceae) resulted in a 2 to 3-fold increase in omega-3 fatty acids in layer eggs, improving their nutritional quality (Nitsan et al., 1999).

The levels of certain heavy metals in dairy cattle feed can be potentially toxic (Miller, 1979) and maximum tolerable dietary levels (MTDL) for Al, As, Br, Cd, F, Hg, Mo, Ni, Pb and V have been promulgated (NRC, 1989). The MTDL for elements measured in algae in this study, based on soluble salts of high bioavailability, include: Al (1000 ppm), Cu (80 ppm), Cd (0.5 ppm), Fe (1000 ppm), Mo (10 ppm), Mn (1000 ppm), Ni (50 ppm), Pb (30 ppm) and Zn (500 ppm) (Miller, 1979; NRC, 1989). Only MTDL for Al, Cu, and Fe are exceeded by levels found in benthic algae in this study. Algae grown from the UF digested manure contained higher levels of Al (2170 ppm) and Cu (106 ppm), while algae grown on USDA undigested and digested manure exceeded MTDL for Fe (1790 and 1060 ppm, respectively). Benthic freshwater algae have been shown to accumulate heavy metals (Pyatt et al., 1993; Vymazal, 1990) to levels far above levels found in their aqueous environment. Yet, Pyatt et al. (1993) suggested that Pb and Zn were mainly absorbed on algae surfaces since they found that washing samples of *Ulothrix* and *Spirogyra* from mine tailing leachates reduced Pb and Zn levels by a factor of over 1000. Since dried algae would only constitute a portion of the protein levels in feed rations, and since these potentially toxic elements are not likely to be in a highly soluble or available form, these higher levels are not expected to reduce feed value. However, levels and bioavailability of these elements, as well as methods for reducing them in freshwater algal biomass, warrant further investigation.

4.2. Benthic algae as soil amendment

In addition to the use of harvested algae as a feed material, there is also the potential to use the algae as a biofertilizer or soil amendment either for crop production or for exporting nutrients off-farm through sale of algal biomass as a fertilizer or soil-conditioning product. Since harvested algae concentrate the nutrients in a smaller volume than in the original manure wastewater, this facilitates nutrient storage and transportation costs. The harvested algae in this study averaged 5.8% TS as they were scraped from the screen. Thus, on a wet basis, the UF digested manure-grown algae had a TN content of 4110 mg kg^{-1}, while the diluted manure as fed had a TN content of 89 mg L^{-1}, suggesting a 46-fold concentration of N. For P, this concentrating effect was 85-fold. If the algae could be dewatered to 40% TS, the resulting fertilizer product would have a TN content of 2.8%, which is comparable or higher than levels in organic fertilizer products made of composted manure. As the algal product would likely have lower pathogen levels than raw manure, it might command a higher return than existing organic fertilizer products.

No known studies have evaluated benthic algae as a fertilizer, but *Spirulina* and other blue-green algae have been evaluated for improving the cultivation of rice (Venkataraman, 1986). A mixture of *Spirulina* and fly ash was found to enhance the growth of rice over conventional fertilization (Banerjee and Deb, 1996). Since benthic freshwater algae contain high levels of plant nutrients, they should also be amenable to fertilizer use.

The soil-conditioning properties of eukaryotic algae have received more attention (Metting, 1988) and are attributed to the coating of soil particles with polysaccharides or to the stimulation of soil bacteria and fungi to excrete soil-conditioning agents. Improving soil aggregate stability, which has been experienced as a result of algae application, can minimize erosion and optimize aeration, water movement, root development, fertilizer use and water holding capacity. Applications of *Chlamydomonas mexicana* onto sandy soils through center-pivot sprinklers over a period of 2–4 years has significantly improved aggregate stability, increased silage yield, reduced water consumption, and reduced energy use associated with pumping (Metting, 1987). This improved soil conditioning was not observed on non-irrigated croplands or on clayey soils. Both *Microspora amoena* and *Ulothrix variabilis* were shown to reduce wind and water erosion and increase moisture holding capacity of sands (Mackenzie and Pearson, 1979). Dairies with irrigated croplands on sandy soils might benefit from long-term applications of benthic algal slurries, which could decrease irrigation demand, improve nutrient retention, and reduce the potential for groundwater contamination.

4.3. Growth rates and nutrient uptake

Algal growth rates and nutrient uptake values by algae grown on the undigested and anaerobically digested dairy manures are roughly comparable to values obtained using algae grown on treated municipal wastewater and in mesocosm studies (Craggs et al., 1996a; Adey and Loveland, 1998). N and P values for the harvested algal biomass fall within the ranges cited by

Table 5
Summary of dry matter (DM) and protein yields, and nutrient uptake data for algae grown on dairy manures in this study compared to a corn/rye silage rotation and other benthic algae

	Manure source			Corn + rye silage[a]	Other benthic algae[b]
	USDA undigested	USDA digested	UF digested		
N loading (kg ha^{-1} yr^{-1})	2330	2730	3760	nd	5000
P loading (kg ha^{-1} yr^{-1})	584	276	412	nd	5770
DM yield (kg ha^{-1} yr^{-1})	19500	19500	20200	26900	72000
VS (% of DM)	87.9	89.0	86.4	nd	45.2
VS yield (kg ha^{-1} yr^{-1})	17200	17400	17400	nd	32600
Crude protein (% of DM)[c]	31.2	30.8	44.3	8.6	19.3
Crude protein yield (kg ha^{-1} yr^{-1})	6080	6000	8950	2320	13900
N uptake rate (kg ha^{-1} yr^{-1})	973	959	1430	371	2230
P uptake rate (kg ha^{-1} yr^{-1})	503	300	298	69	1440
Hectare requirement for 1000 kg available N[d] yr^{-1} (∼20 dairy cows)[e]	1.03	1.04	0.70	2.70	0.45
Hectare requirement for 418 kg P yr^{-1} (∼20 dairy cows)[e]	0.83	1.39	1.40	6.01	0.29

[a] Calculated from corn silage data and ryegrass forage data in NRCS (1999).

[b] Calculated from Craggs et al. (1996a,b).

[c] Crude protein calculated as 6.25 times TKN or N content on dry matter (DM) basis.

[d] Available N assumed to be 50% of TN.

[e] Calculated from dairy waste characterization data in ASAE (1999) using a 635 kg cow.

Adey (6–9% and 1-2% dry basis, respectively) for nutrient-rich wastewater (Adey and Loveland, 1998). Elemental composition of these manure-grown algae differed from that reported for algae grown on treated municipal wastewater (Craggs et al., 1996b) and differed depending on the source of the manure.

4.4. Nutrient removal

Despite significant nutrient uptake by the benthic algae in this study, significant levels of nutrients remained in the effluents (Table 4). In the case of effluent from algae grown on UF digested manure, 63.0 mg L^{-1} of NO_3–N remained in the effluent. Since the algae grown with UF digested manure were subjected to the highest N loading rate, a significant portion of the N content of the digested manure was nitrified. The phenomenon and mechanism for the inhibition of NO_3-uptake by the preferential assimilation of ammonium is well-documented (Syrett, 1981). It is conceivable that lower N loading rates and/or a longer period between manure feedings could reduce the buildup of NO_3 in BAGC effluents.

5. Conclusion

The growth of benthic algae on undigested and digested dairy manures has the potential to lower the acreage requirements for nutrient uptake and increase protein yield compared to conventional cropping rotations. In Table 5 the results from the current studies are compared with a typical corn/rye silage rotation (NRCS, 1999) and to the results from a full-scale benthic algae system (Craggs et al., 1996a,b). While the corn/rye silage rotation gives a higher dry matter yield than the algae grown on dairy manure, in all three cases the crude protein yield is higher in the algae than in the typical corn/rye rotation. The land requirements for algae production are less than those required for corn/rye on both an N and a P basis, using dairy waste characterization data from ASAE (1999) and assuming a 635 kg cow. Algal growth on UF digested manure would require 26% and 23% of the acreage for equivalent N and P uptake, respectively, compared to a corn/rye rotation (Table 5). The data from the other benthic algae study indicate that even higher levels of nutrient uptake and lower levels of land acreage are possible.

While actual costs for the production and processing of benthic algae are unknown, large-scale production systems (> 1000 m^2) are in place for tertiary treatment of sewage (Craggs et al., 1996a,b). Completely replacing conventional cropping systems with benthic algae production may not be practical or cost effective. However, the production of algae from a portion of manure nutrients to replace high-protein feed supplements which are often imported (along with considerable nutrients) onto the farm could potentially link consumption and supply of on-farm nutrients. Combining conventional cropping systems with an algal treatment system could facilitate more efficient crop production and farm nutrient management, allowing dairy operations to be environmentally sustainable on fewer acres. The influence of N loading on algal crude protein levels, the feed value of algal biomass, and the safety of metal and pathogen levels require further investigation. Yet, the potential for high protein yields and nutrient uptake rates justifies consideration of benthic freshwater algae production systems for nutrient recovery from dairy manures.

Acknowledgements

This research was supported by the Florida Agricultural Experiment Station and approved for publication as Journal Series No. R-08140.

References

Adey, W.H., Hackney, J.M., 1989. The composition and production of tropical marine algal turf in laboratory and field experiments. In: Adey, W.H. (Ed.), The Biology, Ecology and Mariculture of *Mithrax spinosissimus* Utilizing Cultured Algal Turfs. The Mariculture Institute, Washington, DC.

Adey, W.H., Loveland, K., 1998. Dynamic Aquaria: Building Living Ecosystems, second ed. Academic Press, San Diego, CA, 498p.

Adey, W., Luckett, C., Jensen, K., 1993. Phosphorus removal from natural waters using controlled algal production. Restor. Ecol. 1 (1), 29–39.

APHA, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Appler, H.N., Jauncey, K., 1983. The utilization of a filamentous green alga (*Cladophora glomerata* (L) Kutzin) as a protein source in pelleted feeds for *Sarotherodon* (tilapia) *niloticus* fingerlings. Aquaculture 30 (1–4), 21–30.

ASAE, 1999. Manure production and characteristics. ASAE D384.1 DEC98. In: ASAE Standards 1999. American Society of Agricultural Engineers, St. Joseph, MI, pp. 663–665.

Banerjee, M., Deb, M., 1996. Potential of fly ash and *Spirulina* combination as a slow release fertilizer for rice field. Cientifica, Sao Paulo 24 (1), 55–62.

Barna, A., Nagy-Toth, F., 1980. Possibility of utilizing thermally polluted waters in cultivating algae. Stud. Cercet. Biol. Ser. Biol. Veg. 32 (1), 47–51.

Bonomo, L., Pastorelli, G., Zambon, N., 1997. Advantages and limitations of duckweed-based wastewater treatment systems. Water Sci. Technol. 35 (5), 239–246.

Bothwell, M.L., 1983. All-weather troughs for periphyton studies. Water Res. 17 (12), 1735–1741.

Boyd, C.E., 1973. Amino acid composition of freshwater algae. Arch. Hydrobiol. 72 (1), 1–9.

Costa, R.H.R., Bavaresco, A.S.L., Medri, W., Philippi, L.S., 2000. Tertiary treatment of piggery wastes in water hyacinth ponds. Water Sci. Technol. 42 (10–11), 211–214.

Craggs, R.J., Adey, W.H., Jenson, K.R., St. John, M.S., Green, F.B., Oswald, W.J., 1996a. Phosphorus removal from wastewater using an algal turf scrubber. Water Sci. Technol. 33 (7), 191–198.

Craggs, R.J., Adey, W.H., Jessup, B.K., Oswald, W.J., 1996b. A controlled stream mesocosm for tertiary treatment of sewage. Ecol. Eng. 6 (1–3), 149–169.

Crawford, J.J.W., Wilkie, A.C., Lincoln, E.P., 1993. Microalgae for nitrogen reduction of anaerobically digested dairy wastewater. In: Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993, Q-433. American Society for Microbiology, Washington, DC, p. 425.

Drenner, R.W., Day, D.J., Basham, S.J., Smith, J.D., Jensen, S.I., 1997. Ecological water treatment system for removal of phosphorus and nitrogen from polluted water. Ecol. Appl. 7 (2), 381–390.

Fallowfield, H.J., Svoboda, I.F., Martin, N.J., 1992. Aerobic and photosynthetic treatment of animal slurries. In: Fry, J.C., Gadd, G.M., Herbert, R.A., Jones, C.W., Watson-Craik, I.A. (Eds.), SGM Symposium 48, Microbial Control of Pollution. Cambridge University Press, Cambridge, UK, pp. 171–197.

Fekete, A., Riemer, D., Motto, H., 1972. Removal of *Rhizoclonium* from a pond and its relationship to dissolved nutrients. Proc. Northeast Weed Sci. Soc. 26, 193–196.

Franklin, S.T., Martin, K.R., Baer, R.J., Schingoethe, D.J., Hippen, A.R., 1999. Dietary marine algae (*Schizochytrium* sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129 (11), 2048–2054.

Hindak, F., Pribil, S., 1968. Chemical composition, protein digestibility and heat of combustion of filamentous green algae. Biol. Plant. (Prague) 10 (3), 234–244.

Ionescu, A., 1970. Productivity of the alga *Ulothrix* in Lacu Sarat and in laboratory cultures. Rev. Roum. Biol. Ser. Bot. 15 (2), 103–109.

Jamieson, G.R., Reid, E.H., 1976. The fatty acid composition of *Ulothrix aequalis* lipids. Phytochemistry 15 (5), 795–796.

Johnson Jr., J.C., Newton, G.L., Butler, J.L., 1991. Recycling liquid dairy cattle waste to sustain annual triple crop production of forages. In: Proceedings of the 28th Annual Florida Dairy Production Conference. Dairy Science Department, University of Florida, Gainesville, FL, pp. 41–50.

Johnston, H.W., 1970. The biological and economic importance of algae, Part 3. Edible algae of fresh and brackish waters. Tuatara 18, 19–35.

Lincoln, E.P., Earle, J.F.K., 1990. Wastewater treatment with microalgae. In: Akatsuka, I. (Ed.), Introduction to Applied Phycology. SPB Academic Publishing, The Hague, pp. 429–446.

Lincoln, E.P., Wilkie, A.C., 1995. Use of filamentous cyanobacteria for nitrogen removal from dairy wastewater. In: Abstracts of the 95th General Meeting of the American Society for Microbiology 1995, Q-291. American Society for Microbiology, Washington, DC, p. 451.

Lincoln, E.P., Crawford, J.J.W., Wilkie, A.C., 1993. *Spirulina* in animal agriculture. Bull. Inst. Oceanogr. (Monaco) 12, 109–115.

Lincoln, E.P., Wilkie, A.C., French, B.T., 1996. Cyanobacterial process for renovating dairy wastewater. Biomass Bioenergy 10 (1), 63–68.

Mackenzie, H.J., Pearson, H.W., 1979. Preliminary studies on the potential use of algae in the stabilization of sand wastes and wind blow situations. Br. Phycol. J. 14 (2), 126.

Metting, B., 1987. Dynamics of wet and dry aggregate stability from a three-year microalgal soil conditioning experiment in the field. Soil Sci. 143 (2), 139–143.

Metting, B., 1988. Micro-algae in agriculture. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), Micro-algal Biotechnology. Cambridge University Press, Cambridge, UK, pp. 288–304.

Miller, W.J., 1979. Dairy Cattle Feeding and Nutrition. Academic Press, New York, 411p.

Nitsan, Z., Mokady, S., Sukenik, A., 1999. Enrichment of poultry products with $\omega 3$ fatty acids by dietary supplementation with the alga *Nannochloropsis* and mantur oil. J. Agric. Food Chem. 47 (12), 5127–5132.

NRC, 1989. Nutrient Requirements of Dairy Cattle, sixth revised ed. update. National Research Council, National Academy Press, Washington, DC, 158p.

NRCS, 1999. National Engineering Handbook Part 651: Agricultural Waste Management Field Handbook. US Department of Agriculture, Natural Resources Conservation Service, Washington, DC.

Nurdogan, Y., Oswald, W.J., 1995. Enhanced nutrient removal in high-rate ponds. Water Sci. Technol. 31 (12), 33–43.

Olguin, E.J., Galicia, S., Camacho, R., Mercado, G., Perez, T.J., 1997. Production of *Spirulina* sp. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. Appl. Microbiol. Biotechnol. 48 (2), 242–247.

Przytocka-Jusiak, M., Blaszczyk, M., Kosinska, E., Bisz-Konarzewska, A., 1984. Removal of nitrogen from industrial wastewaters with the use of algal rotating disks and denitrification packed bed reactor. Water Res. 18 (9), 1077–1082.

Pyatt, F.B., Pyatt, A.J., Mould, N., 1993. Accumulation of lead and zinc from a metalliferous spoil tip stream by the algae *Ulothrix* sp. and *Spirogyra* sp. Int. J. Environ. Stud. 45, 57–59.

Rectenwald, L.L., Drenner, R.W., 2000. Nutrient removal from wastewater effluent using an ecological water treatment system. Environ. Sci. Technol. 34 (3), 522–526.

Singh, C.S., 1970. *Oedogonium obtruncatum* Wittrock as a nutritive source to *Cirrhinus mrigala* (Ham.). Indian J. Exp. Biol. 8 (2), 153–155.

Skadovski, S.N., 1961. Periphyton Communities in the Function of the Bioeliminator. Moscow University Press, Moscow, 363p.

Sladeckova, A., Marvan, P., Vymazal, J., 1983. The utilization of periphyton in waterworks pre-treatment for nutrient removal from enriched influents. In: Wetzel, R.G. (Ed.), Developments in Hydrobiology 17, Periphyton of Freshwater Ecosystems. Dr. W. Junk Publishers, The Hague, pp. 299–303.

Steel, R.G.D., Torrie, J.H., 1980. Independent samples and unequal variances. In: Principles and Procedures of Statistics: A Biometrical Approach, second ed. McGraw-Hill, New York, pp. 106–107.

Syrett, P.J., 1981. Nitrogen metabolism of microalgae. Can. Bull. Fish. Aquat. Sci. 210, 182–210.

Tanner, C.C., Clayton, J.S., Upsdell, M.P., 1995. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—II. Removal of nitrogen and phosphorus. Water Res. 29 (1), 27–34.

USEPA, 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020 (Revised March 1983). US Environmental Protection Agency, Cincinnati, OH.

Venkataraman, L.V., 1986. Blue-green algae as biofertilizer. In: Richmond, A. (Ed.), CRC Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL, pp. 455–471.

Vymazal, J., 1988. The use of periphyton communities for nutrient removal from polluted streams. Hydrobiologia 166 (3), 225–237.

Vymazal, J., 1990. Uptake of heavy metals by *Cladophora glomerata*. Acta Hydrochim. Hydrobiol. 18 (6), 657–665.

Whitford, L.A., 1960. The current effect and growth of fresh-water algae. Trans. Am. Microsc. Soc. 79 (3), 302–309.

Whitford, L.A., Schumacher, G.J., 1961. Effect of current on mineral uptake and respiration by a fresh-water alga. Limnol. Oceanogr. 6 (4), 423–425.

Wilkie, A.C., 2000a. Anaerobic digestion: holistic bioprocessing of animal manures. In: Proceedings of the 1999 Animal Residuals Management Conference. Water Environment Federation, Alexandria, VA, pp. 1–12.

Wilkie, A.C., 2000b. Fixed-film anaerobic digester: Reducing dairy manure odor and producing energy. BioCycle 41 (9), 48–50.

Wilkie, A.C., Riedesel, K.J., Cubinski, K.R., 1995. Anaerobic digestion for odor control. In: Van Horn, H.H. (Ed.), Nuisance Concerns in Animal Manure Management: Odors and Flies. Florida Cooperative Extension, University of Florida, Gainesville, FL, pp. 56–62.

Wood, A., 1987. A simple wastewater treatment system incorporating the selective cultivation of a filamentous algae. Water Sci. Technol. 19 (7), 1251–1254.