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Treatment of Dairy Manure Effluent Using Freshwater Algae: Elemental Composition of Algal Biomass at Different Manure Loading Rates

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The cultivation of algae on nitrogen (N) and phosphorus (P) in animal manure effluents presents an alternative to the current practice of land application. However, the use and value of the resulting algal biomass as a feed or soil supplement depend, in part, on whether the biomass contains any harmful components such as heavy metals. The objective of this study was to determine how the elemental composition of algae changed in response to different loading rates of anaerobically digested flushed dairy manure effluent. Algal biomass was harvested weekly from laboratory-scale algal turf scrubber (ATS) units using four manure loading rates (2, 4, 6, or 9 L m^{-2} day^{-1}) corresponding to daily loading rates of 0.8−3.7 g of total N and 0.12−0.58 g of total P. Mean N and P contents in the dried biomass increased 1.6−1.8-fold with increasing loading rate up to maximums of 6.5% N and 0.84% P at 6 L m^{-2} day^{-1}. Concentrations of Al, Ca, Cu, Fe, Mg, Mn, and Zn showed similar 1.4−1.8-fold increases up to maximums at a loading rate of 6 L m^{-2} day^{-1}, followed by plateaus or decreases above this loading rate. Concentrations of Cd, Mo, and Pb initially increased with loading rate but then declined to levels comparable to those at the lowest loading rate. Concentrations of Si and K did not increase significantly with loading rate. The maximum concentrations of individual components in the algal biomass were as follows (in mg kg^{-1}): 1100 (Al), 9700 (Ca), 0.43 (Cd), 56 (Cu), 580 (Fe), 5.0 (Pb), 2300 (Mg), 240 (Mn), 3.0 (Mo), 14,700 (K), 210 (Si), and 290 (Zn). At these concentrations, heavy metals in the algal biomass would not be expected to reduce its value as a soil or feed amendment.

KEYWORDS: Algal turf scrubber; dairy manure; phytoremediation; algae; composition

INTRODUCTION

Off-farm losses of nutrients from manure during storage in lagoons and subsequent land application can pose a threat to environmentally sensitive watersheds. Cultivating algae on nutrients in animal manure offers an alternative to the current practice of land application. Suspended algae can be cultivated and harvested using wastewater in slowly mixed, shallow raceways (*1*−*5*). Alternatively, attached algae can be grown in rapidly mixed, shallow raceways lined with a suitable attachment surface (*6*−*9*). Both types of systems are highly productive and yield algal biomasses that are potentially valuable as soil amendments or feed supplements (*10*).

Removal of nutrients from raw and anaerobically digested dairy manure using attached algae has been recently studied in laboratory-scale algal turf scrubber (ATS) units (*9*). However, with the exception of N and P, there has been no examination of how the elemental composition of the algal biomass changes as a function of manure loading rate. This is important because use of the algal biomass as a feed or soil supplement depends, in part, on whether the biomass contains any harmful components. Previous research has shown that freshwater algae can accumulate heavy metals to levels far above levels found in their aqueous environment (*11*−*13*). The objective of this study was to determine how the elemental composition of harvested algal biomass changes using different loading rates of anaerobically digested flushed dairy manure.

MATERIALS AND METHODS

Experiments were conducted using anaerobically digested flushed manure effluent from the Dairy Research Unit of the University of Florida in Gainesville, FL, as previously described (*9*). The flushed dairy manure underwent mechanical solids separation and settling prior to being pumped into a 400 m^3 fixed-film anaerobic digester operating at ambient temperature with a 2-day retention time (*14*). All tests were performed on one batch of digested manure effluent that was shipped to Maryland in 200 L barrels and stored at 4 °C. The digested manure effluent concentrations of ammonium-N, nitrate-N, total N (TN), soluble reactive phosphorus, and total P (TP) were 233, <1, 412, 55.8, and 64.5 mg L^{-1}, respectively. The elemental composition of the manure effluent is shown in **Table 1**. Three laboratory-scale ATS units (each

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Table 1. Elemental Composition of Dairy Manure and Dried Algal Biomass Grown Using Four Different Manure Loading Rates

		algal biomass[a] (mg/ kg)					
loading rate (L day^{-1}):		1.90 ± 0.15	3.67 ± 0.43	5.84 ± 0.39	7.82 ± 0.92		
(g of TN day^{-1}):		0.78 ± 0.06	1.48 ± 0.20	2.41 ± 0.16	3.22 ± 0.38		
(g of TP day^{-1}):		0.12 ± 0.01	0.23 ± 0.03	0.38 ± 0.02	0.50 ± 0.06		
element	dairy manure (mg/L)	(n = 5)	(n = 9)	(n = 9)	(n = 9)	daily max loading[b] (mg day^{-1})	recovery in algal biomass at max loading rate[b] (%)
N	412	35200 ± 5000	46400 ± 5200	64800 ± 3500	68600 ± 4000	3400 ± 480	35.9 ± 11.4
K	264	12530 ± 4960	14150 ± 2480	14720 ± 2560	13780 ± 1950	2180 ± 310	11.3 ± 3.2
P	50.4	5290 ± 1350	6570 ± 570	8390 ± 1070	8030 ± 950	420 ± 60	34.3 ± 6.2
Ca	241	6240 ± 1820	6810 ± 1000	9670 ± 3390	8460 ± 1380	1990 ± 300	7.1 ± 1.4
Mg	61.7	1370 ± 50	1580 ± 150	2330 ± 420	1890 ± 200	510 ± 79	5.9 ± 1.1
Al	9.8	610 ± 100	730 ± 180	1060 ± 190	1070 ± 190	80.8 ± 11	23.5 ± 5.0
Fe	4.70	350 ± 40	420 ± 60	580 ± 120	530 ± 100	38.8 ± 5.7	22.7 ± 4.4
Mn	1.84	123 ± 7	149 ± 28	236 ± 52	222 ± 32	15.2 ± 2.3	24.2 ± 5.6
Zn	1.65	176 ± 15	243 ± 87	260 ± 29	289 ± 61	13.6 ± 1.7	33.4 ± 4.6
Si	1.38	212 ± 56	194 ± 40	206 ± 46	210 ± 41	11.4 ± 2.1	25.1 ± 6.8
Cu	0.39	27.8 ± 6.1	41.3 ± 10.6	52.9 ± 7.0	55.7 ± 11.1	3.22 ± 0.52	26.0 ± 4.5
Mo	0.024	2.10 ± 0.30	2.90 ± 0.69	2.05 ± 0.87	1.66 ± 0.27	0.20 ± 0.03	14.2 ± 2.5
Pb	0.010	2.93 ± 0.92	5.00 ± 0.78	2.75 ± 0.59	3.00 ± 0.72	0.082 ± 0.011	68.7 ± 19.8
Cd	0.011	0.29 ± 0.08	0.23 ± 0.07	0.43 ± 0.16	0.31 ± 0.12	0.091 ± 0.008	9.4 ± 3.3

[a] Values are means ± SD of measurements over five to nine harvest cycles. n = number of harvests. [b] Values calculated using a manure loading rate of 9 L m^{-2} day^{-1}.

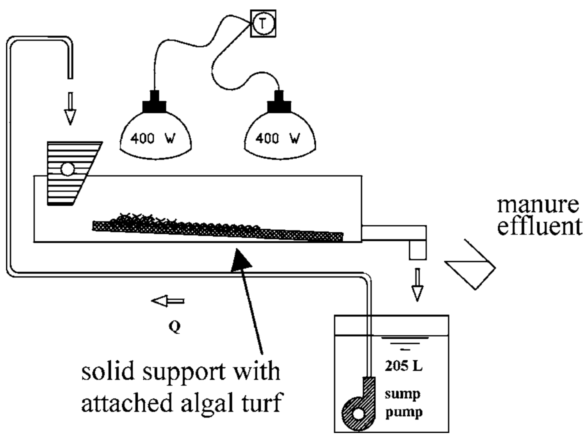

Figure 1. Schematic drawing of a laboratory-scale algal turf scrubber (ATS) unit. Scrubber effluent (205 L) is contained in a plastic drum and continuously recycled through the system using a sump pump with a flow rate (Q) = 100 L min^{-1}. A plastic trough fills and tips over, releasing pulses of effluent that wash over the attached algal turf every 10–15 s before draining back into the plastic drum. Digested dairy manure effluent (2, 4, 6, or 9 L per day) was added daily to the recirculating scrubber effluent.

containing 1 m^2 growing area) were operated in a semicontinuous mode by recycling 205 L of effluent contained in plastic drums and adding manure effluent daily (**Figure 1**). Units were maintained at ambient laboratory temperature (23−26 °C) and illuminated using two 400-W metal halide lights under nearly continuous light (23-h photoperiod). Incident light averaged about 270 (range = 160−460) μmol of photons m^{-2} s^{-1} on two ATS units and 390 (range = 240−633) μmol of photons m^{-2} s^{-1} on a third unit. The ATS units, originally established with algal consortia from a nearby stream in Beltsville, MD, 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* species. To minimize volatilization of ammonia, effluent pH was maintained between 7 and 7.5 by bubbling the system with carbon dioxide or by daily additions of dilute hydrochloric acid. Four different loading rates of manure (2, 4, 6, and 9 L m^{-2} day^{-1}), corresponding to 0.8−3.7 g of TN and 0.12−0.58 g of TP m^{-2} day^{-1}, were tested as described (*9*). Algal biomass was harvested every 3−7 days and dried at 70 °C prior to analysis for total Kjeldahl nitrogen (TKN), TP, and elemental composition using inductively coupled plasma (ICP) analysis (*15*).

RESULTS

ATS units were operated at loading rates of 2, 4, 6, and 9 L m^{-2} day^{-1}. Algal production varied from 5 to 23 g of DW m^{-2} day^{-1} with mean values ranging from 7.6 to 19.1 g of DW m^{-2} day^{-1} at the different loading rates (*9*). The relative content of ash-free dry weight (% DW) for algal biomass grown under all treatments was fairly constant, between 90 and 93%. Mean N and P contents in the dried biomass increased 1.6−1.8-fold with increasing loading rate up to 6.5% N and 0.84% P, at 6 L m^{-2} day^{-1} (**Figure 1**; **Table 1**). Above this loading rate, algal N content increased slightly (to 6.9%) and P decreased slightly (to 0.8%) (*9*). Levels of Al, Ca, Cu, Fe, Mg, Mn, and Zn showed similar 1.4−1.8-fold increases up to maximums at a loading rate of 6 L m^{-2} day^{-1}, followed by plateaus or by changes of <10% above this loading rate. Levels of Mo and Pb initially increased with loading rate but then declined to levels comparable to those at the lowest loading rate. Cd levels decreased slightly at 4 L m^{-2} day^{-1}, increased at 6 L m^{-2} day^{-1}, but then declined to levels comparable to those at the lowest loading rate. Concentrations of Si and K did not increase significantly with loading rate (**Figure 2**; **Table 1**).

The recovery rate for a particular element is a function of algal production and the elements content in the biomass. The absolute amounts of different elements recovered in the algal biomass generally increased with loading rate because of increasing production (not shown). However, percentage recovery for a number of elements (K, P, Ca, Mg, Fe, Mn, Mo, and Cd) peaked at a loading rate of 6 L m^{-2} day^{-1} corresponding to the point of their maximum respective concentrations in the algal biomass (**Table 1**). Individual loading rates and percentage recoveries of different elements in the algal biomass at the highest manure loading rate (9 L m^{-2} day^{-1}) are also shown in **Table 1**. At this loading rate, average recoveries generally varied from values of <10% (Ca, Cd, Mg) to values of 33−36% (N, P, Zn). In contrast, calculated recovery values for Pb were variable and very high relative to recoveries for the other elements. Mean Pb recoveries ranged from 70 to 80% at the two highest loading rates and exceeded 100% at the two lower

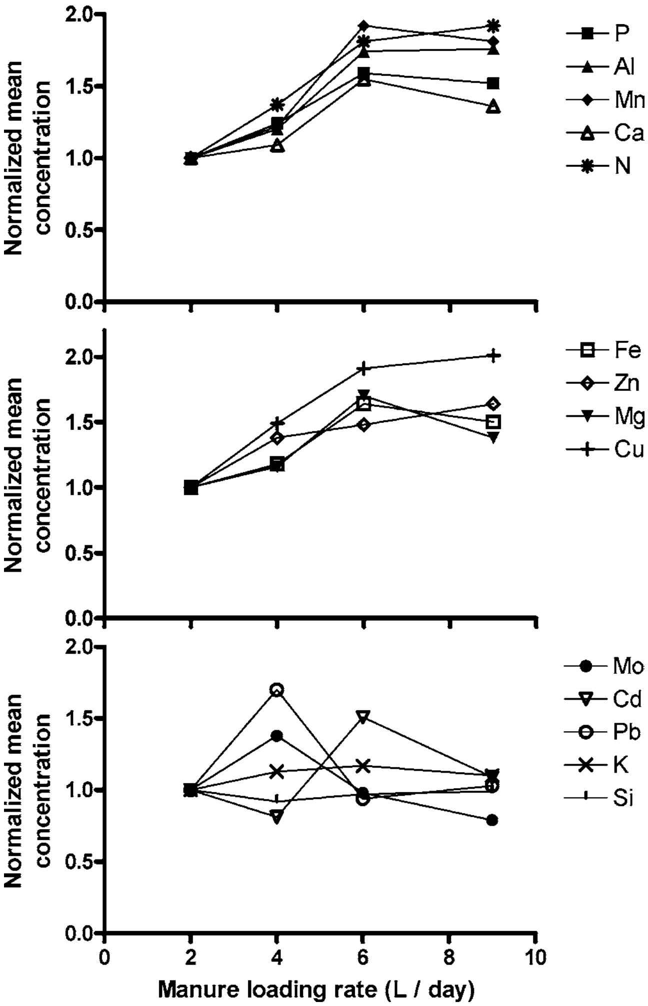

Figure 2. Normalized concentrations of algal biomass constituents as a function of manure loading rate. Constituent values were normalized relative to their values at a manure loading rate of 2 L m^{-2} day^{-1}.

loading rates (not shown). Given these results, it is possible that the ATS units became contaminated from another source of lead during these experiments.

DISCUSSION

The concentrations reported here are generally lower than those from a previous study (*16*) in which algal turfs were grown using a single loading rate of anaerobically digested manure from the University of Florida (corresponding to 1.05 g of TN and 0.12 g of TP m^{-2} day^{-1}) and a much lower light intensity (ranging from 40 to 140 μmol of photons m^{-2} s^{-1}). In that study, use of a lower light intensity resulted in ∼2-fold higher levels of algal N and P (7.1% N, 1.5% P) compared to algal N and P levels (4.0 and 0.7%, respectively) at a comparable manure loading rate (2.5 L m^{-2} day^{-1}; equivalent to 1.05 g of TN and 0.16 g of TP m^{-2} day^{-1}) in this study (*9*). In general, levels of other constituents in the algal biomass are also ∼2−3-fold lower in this study. Exceptions to this are levels of K and Cd (which are roughly equal in both studies), Mo [which is higher in this study (2.9 mg kg^{-1} compared to 1.4 mg kg^{-1} previously)], and Si [which is ∼8-fold lower in this study (200 mg kg^{-1} compared to 1640 mg kg^{-1})]. Unfortunately, we do not have constituent values for the batch of manure used in the previous study. Therefore, it is unclear whether these differences are significant or are simply due to differences in batches of manure.

The maximum tolerable dietary levels (MTDL) in dairy cow feed for elements measured in this study are as follows (in mg kg^{-1}): Al (1000), Cu (100), Cd (0.5), Fe (1000), Mo (5), Mn (1000), Pb (30), and Zn (500) (*17*). These levels are based on the use of highly bioavailable soluble salts of these metals. In this study, only levels of algal Al (with a mean value of 1070 mg kg^{-1} at the highest loading rate) exceeded the MTDL. We have no information about the solubility or bioavailability of any of the constituents in the algal biomass. However, as a potential feed component, dried algal biomass would constitute only a small portion of the total feed (*18*), and thus Al in the product would be unlikely to reduce its value as a feed.

With regard to using the algal biomass as a fertilizer, rough application rates can be calculated on the basis of available N or P fertilizer equivalents. In Maryland, algal production could be expected to operate 9 months of the year at average production values of 15 g of DW algal biomass m^{-2} day^{-1} (equivalent to 150 kg of DW ha^{-1} day^{-1} or 40.5 mt of DW $year^{-1}$). At these loading rates, algal biomass would contain approximately 7% N and 1% P (*9*). The volume of algal biomass from a 100-cow dairy would provide available N fertilizer equivalents for 6 ha of corn at 150 kg ha^{-1} (*19*). As a P fertilizer used for amending soils at 100 kg of P ha^{-1} this volume of algal biomass would support 4 ha of production. At these amendment rates (6.5−10 mt of biomass ha^{-1}), loadings of heavy metals from the algal biomass would be well below regulatory limits (*20*).

ABBREVIATIONS USED

ATS, algal turf scrubber; TP, total phosphorus; TN, total nitrogen; DW, dry weight.

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LITERATURE CITED

(1) Benemann, J. R.; Oswald, W. J. *Systems and Economic Analysis of Microalgae Ponds for Conversion of Carbon Dioxide to Biomass*; Pittsburgh Energy Technology Center: Pittsburgh, PA, 1996.
(2) Craggs, R. J.; Tanner, C. C.; Sukias, J. P.; Davies-Colley, R. J. Dairy farm wastewater treatment by an advanced pond system. *Water Sci. Technol.* **2003**, *48*, 291−297.
(3) Goh, A. Production of microalgae using pig waste as a substrate. In *Algal Biomass Technologies*; Barclay, W. R., McIntosh, R. P., Eds.; Cramer: Berlin, Germany, 1986; pp 235−244.
(4) Lincoln, E. P.; Wilkie, A. C.; French, B. T. Cyanobacterial process for renovating dairy wastewater. *Biomass Bioenergy* **1996**, *10*, 63−68.
(5) Olguin, E. J.; Galicia, S.; Camacho, R.; Mercado, G.; Perez, T. I. Production of *Spirulina* sp. in seawater supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. *Appl. Microbiol. Biotechnol.* **1997**, *48*, 242−247.
(6) Craggs, R. J.; Adey, W. H.; Jenson, K. R.; St. John, M. S.; Green, F. B.; Oswald, W. J. Phosphorus removal from wastewater using an algal turf scrubber. *Water Sci. Technol.* **1996**, *33*, 191−198.
(7) Adey, W. H.; Loveland, K. *Dynamic Aquaria: Building Living Ecosystems*, 2nd ed.; Academic Press: New York, 1998.
(8) Hoffman, J. Wastewater treatment with suspended and nonsuspended algae. *J. Phycol.* **1998**, *34*, 757−763.
(9) Kebede-Westhead, E.; Pizarro, C.; Mulbry, W. W. Production and nutrient removal by periphyton grown under different loading rates of anaerobically digested flushed dairy manure. *J. Phycol.* **2003**, *39*, 1275−1282.

(10) Wilkie, A. C.; Mulbry, W. W. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour. Technol.* **2002**, *84*, 81–91.
(11) Gadd, G. M. Accumulation of metals by microorganisms and algae. In *Biotechnology*; Rehm, H. J., Ed.; VCH: Weinheim, Germany, 1988; pp 401–434.
(12) Genter, R. B. Ecotoxicology of inorganic chemical stress to algae. In *Algal Ecology, Freshwater Benthic Ecosystems*; Stevenson, R. J., Bothwell, M. L., Lowe, R. L., Eds.; Academic Press: San Diego, CA, 1996; pp 403–468.
(13) Vymazal, J. *Algae and Element Cycling in Wetlands*; CRC Press: Boca Raton, FL, 1995.
(14) Wilkie, A. C. *Anaerobic Digester Technology Applications in Animal Agriculture—A National Summit*; Water Environment Federation: Alexandria, VA, 2003; pp 350–354.
(15) APHA. *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; American Public Health Association: Washington, DC, 1995.
(16) Mulbry, W. W.; Wilkie, A. C. Growth of benthic freshwater algae on dairy manures. *J. Appl. Phycol.* **2001**, *13*, 301–306.
(17) NRC. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed.; National Research Council: Washington, DC, 2001.
(18) Franklin, S. T.; Martin, K. R.; Baer, R. J.; Schingoethe, D. J.; Hippen, A. R. Dietary Marine Algae (*Schizochytrium* sp.) Increases Concentrations of Conjugated Linoleic, Docosahexaenoic, and Tranvaccenic Acids in Milk of Dairy Cows. *J. Nutr.* **1999**, *129*, 2048–2054.
(19) Mulbry, W.; Kebede-Westhead, E.; Pizarro, C.; Sikora, L. J. Recyling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresour. Technol.* **2004**, *96* (4), 451–458.
(20) U.S. EPA. *A Plain English Guide to the EPA Part 503 Biosolids Rule*; U.S. Environmental Protection Agency: Washington, DC, 1994.

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