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Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates

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Abstract An alternative to land spreading of manure effluents is to mass-culture algae on the N and P present in the manure and convert manure N and P into algal biomass. The objective of this study was to determine how the fatty acid (FA) content and composition of algae respond to changes in the type of manure, manure loading rate, and to whether the algae was grown with supplemental carbon dioxide. Algal biomass was harvested weekly from indoor laboratory-scale algal turf scrubber (ATS) units using different loading rates of raw and anaerobically digested dairy manure effluents and raw swine manure effluent. Manure loading rates corresponded to N loading rates of 0.2 to 1.3 g TN m^{-2} day^{-1} for raw swine manure effluent and 0.3 to 2.3 g TN m^{-2} day^{-1} for dairy manure effluents. In addition, algal biomass was harvested from outdoor pilot-scale ATS units using different loading rates of raw and anaerobically digested dairy manure effluents. Both indoor and outdoor units were dominated by *Rhizoclonium* sp. FA content values of the algal biomass ranged from 0.6 to 1.5% of dry weight and showed no consistent relationship to loading rate, type of manure, or to whether supplemental carbon dioxide was added to the systems. FA composition was remarkably consistent among samples and >90% of the FA content consisted of 14:0, 16:0, 16:1ω7, 16:1ω9, 18:0, 18:1ω9, 18:2 ω6, and 18:3ω3.

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Introduction

Production of algae as a biofuel feedstock has been the subject of research for at least five decades, and was a focus of the United States Department of Energy's Aquatic Species Program from 1979 to 1995 (Benemann and Oswald 1996; Sheehan et al. 1998). One conclusion of this program was that the most compelling systems would couple an algae-based wastewater treatment system to biofuel production from the resulting biomass. One assumption of this idea is that the entire system would be partially or entirely funded by the value of wastewater treatment. An additional assumption is that the wastewater-grown biomass would contain sufficient levels of fatty acids (FA) to compete with other potential feedstocks. Although there is renewed interest in the production of algae for biofuel, there are few reports of the biofuel potential of wastewater-grown algae. Suspended algae can be cultivated and harvested using wastewater in slowly mixed, shallow (<30 cm deep) raceways (Benemann and Oswald 1996; Craggs et al. 2003; Goh 1986; Lincoln et al. 1996; Olguin 2003). Alternatively, attached algae can be grown in rapidly mixed, very shallow raceways (<2 cm deep) lined with a suitable attachment surface (Adey and Loveland 2007; Craggs et al. 1996; Hoffman 1998; Kebede-Westhead et al. 2003). Both types of systems are highly productive and yield algal biomass that is potentially valuable as soil amendments or feed supplements (Mulbry et al. 2005; Wilkie and Mulbry 2002).

Removal of nutrients from raw and anaerobically digested dairy manure using attached algae has been

recently studied in indoor laboratory-scale and outdoor pilot-scale algal turf scrubber (ATS) units (Kebede-Westhead et al. 2003, 2006). However, there has been no examination of how the FA composition of the algal biomass from these systems (usually dominated by *Rhizoclonium* sp.) changes as a function of manure type or loading rate. Previous research has shown that the green filamentous algae that dominate the ATS systems have low FA content values relative to the FA contents of other algal genera (Benemann and Oswald 1996; Wood 1988). The objective of this study was to determine how the FA content and composition of ATS biomass respond to changes in the type of manure, manure loading rate, and to whether the algae was grown with supplemental carbon dioxide.

Materials and methods

Algal biomass was produced using indoor laboratory-scale algal turf scrubbers (ATS) (Adey and Loveland 2007) with raw and anaerobically digested dairy manure effluent collected from the Dairy Research Unit of the USDA's Beltsville Agricultural Research Center in Beltsville, Maryland, and raw swine manure effluent from a swine finishing operation near Richlands, North Carolina. The characteristics of the respective dairy and swine manure effluents have been described (Wilkie and Mulbry 2002; Kebede-Westhead et al. 2006). Laboratory-scale ATS units containing a 1 m^2 growing area were operated in a semi-continuous mode by recycling 205 L effluent at 55 L min^{-1}, adding manure effluent daily and adding distilled water as needed to compensate for water lost by evaporation. ATS units were maintained at ambient laboratory temperature (18–28°C), and illuminated using two 400 W metal halide lights under nearly continuous light (23:1 h light-dark cycle). Incident light averaged 390 (range 240–633) μmol photons m^{-2} s^{-1}. Manure loading rates (typically 0.3–2.0 L day^{-1}) corresponded to approximately 0.3–2.0 g total nitrogen (TN) m^{-2} day^{-1} as previously described (Kebede-Westhead et al. 2003, 2006).

Algal biomass was also produced from 2003 to 2006 using four 30 m^2 outdoor pilot-scale ATS raceways. Two raceways were constructed at a 1% slope and two were constructed at a 2% slope. Each raceway consisted of a 1× 30 m section of 0.75 mm landfill liner (Gundle Linings Technology, Houston, Texas) covered with 6 mm mesh nylon netting (Apex Mills, Inwood, New York), a 3,700 L underground concrete sump at the base of the raceway, a tipping trough at the top of the raceway, and a submerged water pump in the sump to deliver a flow rate of 93 L min^{-1}. The recirculating effluent consisted of fresh water (untreated well water or chlorinated drinking water) and daily additions of raw or anaerobically digested dairy manure effluent (typically 10–20 L day^{-1}) from the USDA's Dairy Research Unit in Beltsville, Maryland. Although rainwater was usually sufficient to replenish water lost to evaporation, additional fresh water was added as needed to maintain an effluent volume of 3,500 L.

Both indoor and outdoor systems were dominated by the filamentous green algae *Rhizoclonium hieroglyphicum* (C. A. Agardh) Kutz. Wet algal biomass was harvested weekly using a wet/dry vacuum, dewatered by sieving harvested material through 2 mm mesh nylon netting (Aquatic Ecosystems, Apopka, Florida) to approximately 10% solids content, then air dried for approximately 48 h using electric fans to approximately 90% solids content. Dried biomass was initially ground in a Wiley Mill to pass a 3 mm sieve and stored in sealed plastic bags at 20–25°C. For crude fat and FA analyses, aliquots of dried biomass were ground to pass a 0.5 mm sieve and stored in sealed plastic bags as above.

Extraction of crude fat from ATS biomass

Crude fat content was extracted from algal samples using a Dionex 200 accelerated solvent extraction (ASE) system (Dionex Corporation, Salt Lake City, Utah). Dried algal samples (2.5 g) were mixed with 1 g diatomaceous earth and 30 g Ottawa sand (20–30 mesh) prior to being loaded into 33 mL sample cells. After the sample mix was loaded, Ottawa sand was used to fill any extra space in the cell. Extraction conditions for the ASE were: 5 min preheat; pressure, 1,500 psi (10.3 MPa); temperature, 120°C; heat time, 5 min; flush volume, 50% of cell volume; purge time, 60 s; static cycles, 1; solvent (compartment A); chloroform: methanol (2:1, v/v) (Dionex 2004; Luthria et al. 2004; Schafer 1998). The resulting extracts (typically 30 mL) were stored at 4°C prior to removing aliquots for crude fat and FA analysis. The percentage crude fat content was determined gravimetrically by transferring 12 mL aliquots of each extract to pre-weighed vials and evaporating the solvent under a stream of nitrogen. Results are reported on a dry weight (DW) basis. For comparison to other studies that report FA content as a percent of ash free dry weight, the ash content of ATS algae grown using the indoor lab scale system was approximately 10% (the range was 9–11%). The ash content of algae grown using the outdoor pilot scale ATS system was approximately 15% (the range was 13–17%).

FA analysis

Fatty acid methyl esters (FAME) were prepared and analyzed using the MIDI system (Microbial ID, Newark, Delaware). Aliquots (0.25 mL) of crude extracts were dried under a stream of nitrogen, then saponified for 30 min at

Table 1 Means and mean comparisons of fatty acid (FA) content [% dry weight (DW)] of algal biomass from indoor laboratory scale and outdoor pilot-scale algal turf scrubbers (ATS) grown using different manure effluents with and without carbon dioxide supplementation

	Raw swine effluent	Raw dairy effluent	Raw dairy effluent	Digested dairy effluent	Digested dairy effluent
	No added $C0_2$	No added $C0_2$	Added $C0_2$	No added $C0_2$	Added $C0_2$
Laboratory-scale units[c]	0.83 b	0.90 b	1.19 a	0.75 b	0.86 b
Pilot-scale units[d]			0.91		0.89

Means with different letters are different at the 0.05 significance level
[c] Mean comparisons were made at the average loading rate (1.14 g TN m^{-2} day^{-1})
[d] Mean comparisons were made at the average loading rate (0.57 g TN m^{-2} day^{-1})

100°C using 1 mL MIDI Reagent 1 (45 g NaOH, 150 mL H_2O, 150 mL methanol). After cooling, 2 mL MIDI Reagent 2 (325 mL 6 *N* HCl, 275 mL methanol) were added and the solution heated at 80°C for 10 min to methylate the fatty acids. The fatty acid methyl esters were extracted with 1.25 mL MIDI Reagent 3 (1:1 hexane: methyl *tert*-butyl ether) for 10 min. The organic phase was washed with 3 mL MIDI Reagent 4 (10.8 g NaOH, 900 mL H_2O) and then transferred to a gas chromatography vial.

FAME analysis was performed using an Agilent 6890 gas chromatograph with autosampler, split-splitless inlet, flame ionization detector, and Ultra 2 column (25 m long×0.2 mm I.D.×0.33 μm film thickness) (Agilent Technologies, Palo Alto, CA). The carrier gas was hydrogen at a constant pressure of 9.7 psi. A 100:1split injection was used. The injector was held at 250°C while the detector was kept at 300°C. The oven was at 170°C initially, then ramped to 300°C at 5°C/min, and held at 300°C for 10 min. The system was controlled with Chemstation (Agilent) and Sherlock (MIDI) software. The MIDI EUKARY method was used to identify the FAMEs. Palmitic acid (16:0) (Sigma) was used as an external standard for FAME quantitation. The standard was derivitized using the MIDI protocol and analyzed by gas chromatography under the same conditions described above.

Statistical analysis

The FA content data were analyzed separately for indoor laboratory-scale and outside pilot-scale treatments. Since nitrogen loading rates were not equal between manure treatments, FA content data was analyzed as one-factor general linear covariance models using PROC MIXED (SAS Institute 2004) with treatment (manure type with and without supplemental carbon dioxide) as the factor and loading rate the covariate. For both sets of data the equal slopes model was found to be sufficient. The assumptions of the general linear model were met. For indoor laboratory-scale results, treatment mean comparisons were made at an average loading rate of 1.14 g TN m^{-2} day^{-1} (Table 1). Since treatment was statistically significant for laboratory scale results, mean comparisons were done with Sidak adjusted *P*-values to hold the experiment-wise error at 0.05. For outdoor pilot-scale results, treatment mean comparisons were made at an average loading rate of 0.57 g TN m^{-2} day^{-1}.

Results

Algal and FA productivity results with indoor ATS units

Indoor lab scale ATS units were operated at three loading rates of raw swine, raw dairy, and anaerobically digested dairy manure effluents. Mean FA content ranged from 0.6 to 1.3% of DW and generally decreased with increasing manure loading rate (Tables 2, 3). Since nitrogen loading rates were not equal between manure treatments, statistical comparisons of the treatment means were made at an average loading rate of 1.14 g TN m^{-2} day^{-1}. At this loading rate, the mean FA content of algae grown using raw manure supplemented with carbon dioxide was statistically higher (F-value=8.68, *P*-value<.0001, df=4), than the FA contents of algae grown using the four other treatments (Table 1). FA productivity from algae grown with swine manure was notably lower than from algae grown with dairy manure and this was largely due to the relatively low productivity values of swine manure-grown algae (Tables 2, 3). There was no effect of carbon dioxide supplementation on algal productivity or FA productivity with algae grown with digested dairy manure.

Algal and FA productivity results with outdoor pilot scale ATS units

Outdoor pilot scale ATS units were operated at four different loading rates of raw dairy manure effluent and two loading rates of anaerobically digested dairy manure effluent. Results were generally comparable to those from algae grown in lab scale ATS units. FA content ranged from 0.6 to 1.5% of DW with the highest value corresponding to a relatively low loading rate (0.6 g TN m^{-2} day^{-1}) of raw dairy manure (Table 4). There was no effect on algal productivity, FA content, or FA productivity when pilot

Table 2 Fatty acid content and production rate in dried algal biomass from indoor laboratory scale ATS grown using different loading rates of raw dairy or raw swine manure effluents with and without carbon dioxide supplementation

	Raw swine effluent			Raw dairy effluent			Raw dairy effluent		
	No added carbon dioxide			No added carbon dioxide			Added carbon dioxide		
Loading rate (g TN m^{-2} day^{-1})	0.24±0.01	0.62±0.01	1.30±0.01	0.33±0.01	1.60±0.01	2.30±0.01	0.33±0.01	1.60±0.01	2.30±0.01
Algal productivity (g DW m^{-2} day^{-1})	6.8 ±0.8	9.2±0.5	10.7±2.5	8.3±2.0	21.3±2.4	18.6±1.3	8.8±1.2	20.4±2.5	17.9±2.4
Crude fat content (% of DW)	9.3±2.1	9.3±0.2	8.6±0.1	5.3±0.4	5.5±0.4	7.0±0.7	5.3±1.4	5.4±0.4	7.5±0.2
FA content (% of DW)	1.1±0.2	0.9±0.1	0.7±0.1	1.1±0.1	0.7±0.2	0.8±0.1	1.3±0.2	1.0±0.2	1.2±0.1
FA productivity (mg FA m^{-2} day^{-1})	78±18	86±4	72±18	89±22	156±20	151±14	83±19	174±20	210±29

Values are the means±SD of three samples from different weekly harvests

scale ATS raceways were supplemented with carbon dioxide (results not shown). Since nitrogen loading rates were not equal between manure treatments, statistical comparisons of the treatment means were made at an average loading rate of 0.57 g TN m^{-2} day^{-1}. At this loading rate, there was no statistical difference (F-value= 0.02, *P*-value= 0.8771, df=1) between the mean FA contents of algae grown using raw or digested dairy manure effluents (Table 1). With regard to seasonal effects on FA content, at low loading rates (<1 g TN m^{-2} day^{-1}) there were no consistent differences in algal FA content in samples harvested in the spring (April), summer (July), fall (September) or winter (December) from 2003 to 2006 (results not shown). We conducted high loading rate experiments (>1 g TN m^{-2} day^{-1}) only in the spring of 2004. We do not have comparable results from other seasons.

FA composition

Fatty acid composition was remarkably consistent among samples; >90% of the FA content consisted of 14:0, 16:0, 16:1ω7, 16:1ω9, 18:0, 18:1ω9, 18:2 ω6, and 18:3ω3. Results from indoor ATS units using raw swine and raw and digested dairy manure revealed differences in the relative amounts of 16:0, 18:1w9, and 18:3ω3 (Table 5). In swine manure grown algae, the FA 16:0 accounted for 43% of total FA and this value was not affected by manure loading rate. In algae grown with raw or digested dairy manure, the FA 16:0 accounted for 31–38% of total FA and this value was affected neither by manure loading rate nor by whether the system was supplemented with carbon dioxide (Tables 5, 6). Algae grown with swine manure had lower amounts of 16:1ω5, 16:1ω7, 16:1ω9 compared to algae grown with raw or digested dairy manure. In contrast to the consistent values for other FA, values for 18:1ω9 and 18:3ω3 were highly variable among samples from the lab scale ATS.

Results from outdoor ATS using raw and digested dairy manure were generally comparable to values from indoor ATS, but showed higher values of 18:0 and lower values of 18:1ω9 (Tables 5, 6, 7). FA composition values were not affected by manure loading rate (Table 7), nor were they affected by supplemental carbon dioxide (not shown). With regard to seasonal effects on FA composition, as noted above we have few results for biomass grown at high loading rates (>1 g TN m^{-2} day^{-1}). However, at lower loading rates there were no consistent differences in algal

Table 3 Fatty acid content and production rate in dried algal biomass from indoor laboratory scale ATS grown using different loading rates of anaerobically digested dairy manure effluent with and without carbon dioxide supplementation

	Digested dairy effluent			Digested dairy effluent		
	No added carbon dioxide			Added carbon dioxide		
Loading rate (g TN m^{-2} day^{-1})	0.38±0.01	1.30±0.01	1.56±0.01	0.38±0.01	1.30±0.01	1.56±0.01
Algal productivity (g DW m^{-2} day^{-1})	10.5±0.8	13.9±0.8	17.3±2.7	10.3±0.3	16.6±2.3	21.0±3.4
Crude fat content (% of DW)	4.2±0.9	6.9±0.2	6.4±0.2	3.5±0.1	7.7±0.3	6.0±1.3
FA content (% of DW)	0.7±0.1	0.6±0.1	0.9±0.3	0.8±0.1	1.0±0.2	0.8±0.1
FA productivity (mg FA m^{-2} day^{-1})	69±11	86±22	150±22	72±10	138±51	162±37

Values are the means±SD of three samples from different weekly harvests

Table 4 Fatty acid content and production rate in dried algal biomass from outdoor pilot scale ATS grown using different loading rates of raw and anaerobically digested dairy manure effluent

	Raw dairy effluent				Digested dairy effluent	
	(n=5)[a]	(n=5)	(n=4)	(n=5)	(n=7)	(n=5)
Loading rate (g TN m^{-2} day^{-1})	0.39±0.08	0.63±0.10	0.93±0.12	1.63±0.25	0.43±0.09	0.68±0.06
Algal productivity (g DW m^{-2} day^{-1})	5.3±3.1	8.3±2.3	11.6±4.0	14.6±4.2	4.9±2.7	7.6±2.7
Crude fat content (% of DW)	6.5±0.6	9.9±1.1	6.7±0.6	8.0±1.2	8.0±1.4	6.0±1.2
FA content (% of DW)	0.62±0.11	1.47±0.13	0.63±0.08	0.85±0.15	1.02±0.21	0.75±0.21
FA productivity (mg FA m^{-2} day^{-1})	27±15	106±20	63±22	107±31	47±35	46±16

Values are the means±SD of four to seven samples from different weekly harvests
[a] Number of samples

FA composition in samples harvested in the spring (April), summer (July), fall (September) or winter (December) from 2003 to 2006 (results not shown).

Discussion

The fatty acid composition results for manure grown ATS biomass are in broad agreement with FA profiles reported previously for a variety of Chlorophytes (reviewed by Wood 1988; Benemann and Oswald 1996). The ATS profiles are primarily composed of 16:0, 16:1 (ω7 and ω9), 18:1 ω9, 18:2ω6, and 18:3 ω3. There were no significant amounts of FA above C18. Values for fatty acid content are less commonly reported than for fatty acid composition, but values for the ATS biomass (roughly 1% of DW) are on the very low end of values reported for nitrogen sufficient cultures of chlorophytes such as *Chlorella* and *Scenedesmus* (Piorreck and Pohl 1984; Shifrin and Chisholm 1981). Benemann and Oswald (1996) provide an excellent discussion of algal nitrogen status and algal lipid content. There have been no previous reports describing the lipid or FA content of ATS biomass grown using other nutrient inputs or under different environmental conditions. Thus, we have no information whether these low FA content values are indicative of ATS biomass in general or are specific to manure effluent-grown ATS biomass.

Recent cost estimates of an on-farm ATS treatment system to treat dairy manure effluent have used productivity

Table 5 Fatty acid composition in dried algal biomass from indoor laboratory scale ATS grown using different loading rates of raw dairy or raw swine manure effluents with and without carbon dioxide supplementation

Loading rate (g TN m^{-2} day^{-1})	Raw swine effluent			Raw dairy effluent			Raw dairy effluent		
	No added carbon dioxide			No added carbon dioxide			Added carbon dioxide		
	0.24	0.62	1.30	0.33	1.60	2.30	0.33	1.60	2.30
14:0	4.4±1.5	7.3±1.2	5.8±1.2	6.6±1.2	7.1±1.5	6.6±0.5	5.2±1.3	4.8±0.9	5.5±1.0
14:1ω5	nd[a]	nd	nd	nd	2.6±3.1	0.7±1.3	nd	nd	1.6±0.9
15:0	1.7±0.1	1.7±1.6	1.9±0.5	2.2±0.1	3.5±1.1	3.9±0.6	2.3±0.8	3.6±0.6	3.5±1.2
16:0	43.0±4.8	42.5±7.5	43.5±0.4	34.3±3.2	32.5±5.4	33.7±5.3	32.8±0.8	32.2±0.8	31.0±1.8
16:1ω9	1.7±2.9	1.9±3.2	1.8±3.0	3.3±0.9	10.8±4.0	7.6±0.6	2.8±2.0	7.6±3.6	10.2±1.2
16:1ω7	4.0±0.8	7.6±1.0	10.0±3.1	21.7±3.0	11.8±2.5	13.0±3.4	15.4±7.2	9.5±3.5	8.9±1.0
16:1ω5	nd	nd	nd	2.2±0.8	1.1±1.0	0.7±1.1	2.6±1.3	1.8±1.0	1.2±0.6
16:2ω6	2.0±0.2	0.6±1.1	nd	0.3±0.5	1.3±1.2	2.1±0.2	0.7±0.9	2.1±0.3	1.5±0.3
18:0	3.6±0.3	5.5±1.0	6.8±0.6	7.9±1.3	6.2±2.3	6.4±0.9	7.3±3.8	5.6±1.0	5.3±1.2
18:1ω9	12.0±9.9	9.9±15.2	3.8±4.6	4.1±6.0	2.8±4.8	nd	16.3±17.2	1.9±3.3	1.6±0.2
18:3ω3	14.1±13.9	13.9±12.1	17.1±5.0	10.9±9.6	13.0±11.6	16.8±3.4	6.7±11.6	19.1±7.7	19.3±4.1
18:2ω6	10.3±1.6	7.5±0.7	6.8±0.3	4.3±0.9	5.3±1.5	5.6±0.4	4.8±1.2	8.3±2.2	7.1±1.9
20:1ω11/20:ω12[b]	nd	0.2±0.4	0.6±1.0	nd	nd	0.9±1.5	0.7±1.2	0.6±1.1	1.1±0.9

Values are the means±SD of three samples from different weekly harvests
[a] Not detected or <0.2%
[b] These FA co-migrated within a single GC peak and could not be resolved using our method

Table 6 Fatty acid content (% of total FA) of dried algal biomass from indoor laboratory scale ATS grown using different loading rates of anaerobically digested dairy manure effluent with and without carbon dioxide supplementation

	Digested dairy effluent			Digested dairy effluent		
	No added carbon dioxide			Added carbon dioxide		
Loading rate (g TN m^{-2} day^{-1})	0.38±0.01	1.30±0.01	1.56±0.01	0.38±0.01	1.30±0.01	1.56±0.01
14:0	6.4±0.3	8.0±1.0	7.3±1.5	5.5±1.0	6.8±1.4	6.7±1.4
14:1ω5	nd[a]	nd	nd	nd	1.6±1.4	0.6±1.1
15:0	2.0±1.8	2.4±2.1	2.6±1.0	2.1±1.1	2.7±0.8	2.8±0.9
16:0	32.6±2.0	38.2±3.7	35.6±1.3	33.5±1.2	33.9±0.5	37.5±6.0
16:1ω9	6.2±0.6	7.0±0.9	4.7±2.3	3.8±2.3	7.2±0.7	7.6±2.1
16:1ω7	19.8±1.8	9.0±0.3	11.2±0.1	14.3±3.4	11.4±1.9	10.8±1.6
16:1ω5	nd	0.3±0.6	nd	2.0±1.0	0.9±1.0	1.0±1.6
16:2ω6	2.3±1.1	4.0±2.1	4.2±0.8	0.2±0.4	1.2±1.0	1.2±2.1
18:0	3.7±0.6	4.5±0.8	3.6±1.1	3.5±1.0	4.7±0.7	3.9±0.2
18:1ω9	0.5±0.8	6.3±6.1	12.5±8.9	20.7±17.0	11.9±9.1	4.1±7.1
18:3ω3	19.4±1.5	12.4±7.3	6.9±12.0	6.1±10.5	8.0±9.9	14.9±12.9
18:2ω6	7.2±1.0	8.0±1.2	8.6±0.7	5.6±2.0	6.7±0.9	7.1±0.3
20:1ω11/20:1ω12[b]	nd	nd	nd	0.9±1.5	nd	nd

Values are the means±SD of three samples from different weekly harvests
[a] Not detected or <0.2%
[b] These FA co-migrated within a single GC peak and could not be resolved using our method

values extrapolated from both laboratory and pilot scale results (Mulbry et al. 2005; Pizarro et al. 2006). Extrapolated pilot-scale ATS values (average productivity of 10 g DW m^{-2} day^{-1} for 270 days per year) are equivalent to a yearly productivity value of 27,000 kg DW algal biomass ha^{-1} at a loading rate of 2,700 kg TN, 400 kg TP ha^{-1}. Using these values, the estimated yearly operational costs of dried ATS biomass is US \$1.30 kg^{-1}. This estimate does not include any value for the algal biomass nor for any treatment value. Although US \$1.30 kg^{-1} compares very favorably with a recent cost estimate of US \$32 kg^{-1} for microalgal biomass grown in outdoor photobioreactors (Molina-Grima et al. 2003), for ATS biomass with 1% FA content, it is equivalent to a unacceptably high feedstock cost of US \$130 kg^{-1} FA. Within the context of reducing nutrient inputs in sensitive watersheds such as the Chesapeake Bay,

Table 7 Fatty acid content (% of total FA) of dried algal biomass from outdoor pilot scale ATS grown using different loading rates of raw and digested dairy manure effluents

	Raw dairy effluent				Digested dairy effluent	
	(*n*=5)[a]	(*n*=5)	(*n*=4)	(*n*=5)	(*n*=7)	(*n*=5)
Loading rate (g TN m^{-2} day^{-1})	0.39±0.08	0.63±0.10	0.93±0.12	1.63±0.25	0.43±0.09	0.68±0.06
14:0	4.1±0.7	2.1±0.5	3.4±0.4	3.3±0.5	2.6±1.5	5.0±0.6
15:0	2.8±0.9	1.8±0.9	3.3±1.3	2.8±0.9	0.6±0.7	2.1±1.6
16:0	29.8±1.6	25.6±4.3	31.0±1.4	33.1±3.6	25.9±2.9	38.8±5.9
16:1ω9	nd[b]	1.7±2.0	3.7±0.4	3.5±0.6	nd	2.3±2.1
16:1ω8	1.5±3.1	2.1±2.5	nd	4.2±3.7	12.3±4.2	1.1±2.4
16:1ω7	15.8±2.6	10.8±2.1	13.9±3.2	14.5±2.1	7.4±2.9	11.4±2.5
16:2ω6	nd	1.8±0.4	1.8±0.4	2.3±0.5	3.4±0.4	2.3±1.5
17:0	nd	0.4±0.9	nd	0.4±0.9	nd	nd
18:0	23.1±3.8	8.7±4.5	14.9±7.9	9.0±2.8	3.0±1.1	5.0±1.3
18:1ω9	1.0±1.9	2.4±3.9	4.3±5.1	2.1±3.2	1.4±2.4	19.5±4.7
18:3ω3	17.8±0.6	28.0±7.6	12.0±1.8	15.8±6.0	29.2±2.4	nd
18:2ω6	3.6±0.4	0.2±0.4	nd	5.5±0.7	8.9±0.5	8.1±2.2

Values are the means±SD of four to seven samples from different weekly harvests
[a] Number of samples
[b] Not detected or <0.2%

ATS treatment costs of roughly US \$11 kg^{-1} N compare very favorably with the costs of other agricultural nutrient management practices (Chesapeake Bay Foundation 2004). If the nutrient treatment value is included in the cost analysis, then the net biomass costs could be negligible. However, even under this scenario, it seems unlikely that this low FA content biomass would successfully compete with other feedstocks (Chisti 2007).

In addition to biodiesel production, a variety of algae-based bioenergy strategies have been proposed, including low temperature gasification, direct incineration and fermentation (Li et al. 2007; Minowa and Sawayama 1999; Sawayama et al. 1999; Chen and Oswald 1998). Aresta and coworkers have developed life cycle analysis (LCA) computing software to evaluate energy production from different processes and algal feedstocks (Aresta et al. 2005). Ultimately, such assessments will be essential in determining the best use for wastewater-grown biomass.

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