Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock

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Abstract: Algae grown on wastewater media are a potential source of low-cost lipids for production of liquid biofuels. This study investigated lipid productivity and nutrient removal by green algae grown during treatment of dairy farm and municipal wastewaters supplemented with CO_2. Dairy wastewater was treated outdoors in bench-scale batch cultures. The lipid content of the volatile solids peaked at Day 6, during exponential growth, and declined thereafter. Peak lipid content ranged from 14–29%, depending on wastewater concentration. Maximum lipid productivity also peaked at Day 6 of batch growth, with a volumetric productivity of 17 mg/day/L of reactor and an areal productivity of 2.8 $g/m^2/day$, which would be equivalent to 11,000 L/ha/year (1,200 gal/acre/year) if sustained year round. After 12 days, ammonium and orthophosphate removals were 96 and >99%, respectively. Municipal wastewater was treated in semicontinuous indoor cultures with 2–4 day hydraulic residence times (HRTs). Maximum lipid productivity for the municipal wastewater was 24 mg/day/L, observed in the 3-day HRT cultures. Over 99% removal of ammonium and orthophosphate was achieved. The results from both types of wastewater suggest that CO_2-supplemented algae cultures can simultaneously remove dissolved nitrogen and phosphorus to low levels while generating a feedstock potentially useful for liquid biofuels production.

DOI: 10.1061/(ASCE)EE.1943-7870.0000129

CE Database subject headings: Biomass; Wastewater management; Nutrients; Carbon dioxide.

Introduction

Biofuels produced from plants have the potential to replace a significant fraction of our fossil fuel needs with a renewable alternative (Perlack et al. 2005). However, concern has grown that the use of food crops for production of ethanol, biodiesel, or other renewable fuels will increase food prices while having little impact on greenhouse gas emissions (Fargione et al. 2008). Prior work, in particular the Aquatic Species Program sponsored by the U.S. Department of Energy, suggested that algae are capable of producing oil suitable for conversion to biodiesel with an areal productivity 20–40 times that of oilseed crops, such as soy and canola (Sheehan et al. 1998). However, an economic study of such processes (Benemann and Oswald 1996) suggested that large-scale algae cultivation solely for biofuel production was not economical, and the writers reemphasized the integration of biofuels production and wastewater treatment with CO_2 supplementation, as first suggested by Oswald and Golueke (1960). In particular, assimilation of wastewater nutrients by algae followed by algae harvesting via sedimentation were considered potentially practical and economical approaches to biofuel production.

Use of algae for municipal wastewater treatment in ponds is well established (Oswald et al. 1953; Oswald 2003), and algae-based treatment of dairy and piggery waste also has been investigated (e.g., Craggs et al. 2004; Kebede-Westhead et al. 2006; Mulbry et al. 2008; An et al. 2003). Algae growth in wastewater treatment ponds contributes to treatment mainly through dissolved oxygen production and nutrient assimilation. However, the carbon:nitrogen and carbon:phosphorus ratios in domestic sewage (C:N 3.5:1; C:P 20:1) and dairy lagoon water (C:N 3:1; C:P 10:1) are low compared to typical ratios in rapidly growing algae biomass (C:N 6:1; C:P 48:1) (Metcalf and Eddy 2003; USDA 1992; Oswald 1960). This dearth of carbon leads to limitations in algae production and incomplete assimilation of wastewater nutrients by algae. The experiments described in the present research overcame the carbon limitation of the wastewaters by addition of CO_2 to the cultures. The effects of this addition on both algae growth and nutrient assimilation were measured. In future applications, CO_2 could be supplied by the flue gas from power plants and other sources. A schematic of one envisioned process is shown in Fig. 1.

CO_2 supplementation of algae cultures to increase productivity has been studied for many years (Burlew 1953), as has the use of flue gas as a CO_2 source for algae culture (Straka et al. 2000). CO_2 supplementation to promote nutrient removal has also been studied briefly in outdoor ponds (Benemann et al. 1980). However, the production of lipids was not measured in these studies.

Lipid content for pure cultures of algae has been reported to range from 1–85%, and the lipids exhibit varying carbon chain lengths, degrees of unsaturation, and polarity [e.g., reviews in

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Note. This manuscript was submitted on June 17, 2008; approved on July 21, 2009; published online on July 23, 2009. Discussion period open until April 1, 2010; separate discussions must be submitted for individual papers. This paper is part of the ***Journal of Environmental Engineering***, Vol. 135, No. 11, November 1, 2009. ©ASCE, ISSN 0733-9372/2009/11-1115–1122/$25.00.

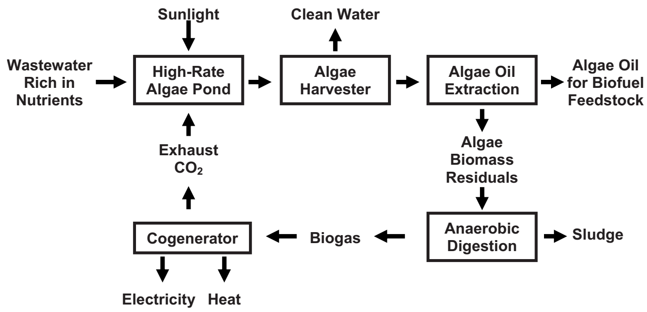

Fig. 1. Simplified process flow diagram envisioned for algae wastewater treatment and liquid biofuel production

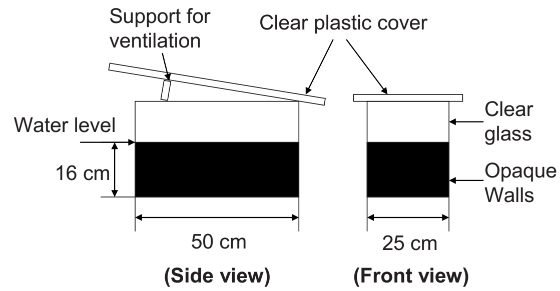

Fig. 2. Outdoor algae growth tanks for batch experiments with dairy wastewater

Chisti (2007), Metting (1996), and Enssani (1987)]. However, the lipid content and, more important, the lipid productivity of polycultures of algae, such as wastewater pond algae, have seldom, if ever, been reported. Further, lipid content, fatty acid profile, and biomass productivity depend on environmental conditions, culturing methods, and growth phase (Thompson 1996; Tsuzuki et al. 1990). In particular, nitrogen limitation increases lipid content in some species (Spoehr and Milner 1949; Leman 1997). However, nitrogen limitation decreases growth rate, which can lead to decreased overall lipid productivity (Shifrin and Chisholm 1981). Benemann and Tillett (1987) investigated this problem, but maximizing lipid productivity remains an outstanding problem.

While a few studies have reported the lipid *content* of waste-grown algae cultures [e.g., 25%, Enssani (1987)], lipid *productivities* for waste-grown polycultures apparently have not been reported previously. The research presented herein was conducted to determine the lipid content and lipid productivity of microalgae grown for nutrient removal from two types of wastewater—dairy and municipal.

Methods

Overview of Experiments

Two sets of experiments were run in parallel to determine algae growth, nutrient removal, and lipid productivity in municipal wastewater and dairy wastewater. The municipal wastewater experiment monitored algae growth under semicontinuous operation for 18 days to study the effects of CO_2 levels and hydraulic residence times (HRTs) on algae growth and nutrient removal. Control cultures with addition of air only (no CO_2) were used to simulate the carbon limitation typical of wastewater ponds and to differentiate the effect of CO_2 addition on productivity. In the dairy wastewater experiment, lipid productivity and nutrient removal were monitored during 15 days of batch growth to study the effect of the growth cycle on lipid content.

Collection and Pretreatment of Wastewater

For the municipal wastewater experiments, 60 L of primary clarifier effluent was collected at the San Luis Obispo, California, municipal wastewater treatment facility. The wastewater was mixed thoroughly and passed through screens with 196-μm openings (commercial house paint filters). The screened wastewater was stored in 4-L HDPE containers at $-10°C$.

For the dairy wastewater experiments, free-stall barn flush water was collected from a storage pond at the 400-head Cal Poly Dairy in San Luis Obispo. In flush operations at this dairy, the flush water is passed through a bar screen and sand trap and then collected in a covered sump. The wastewater is then pumped over a wedge-wire inclined screen, which removes feed and other fine solids, before being discharged to the 0.5-ha storage pond. Wastewater for the research was collected from the storage pond and then treated in an anaerobic digester before being used in algae growth experiments. The 130-L digester was unheated, unmixed, and fed semicontinuously to achieve a 6-week hydraulic residence time.

Municipal Wastewater Experimental Procedures

Eight 1-L Pyrex Roux bottles (Fisher Scientific, Rockford, Ill.) were used as algae growth reactors for the municipal wastewater experiments. Each bottle was placed vertically on a magnetic stirrer and mixed by a polyetrafluoroethylene (PTFE)-coated 2.5-cm magnetic stir bar spinning at approximately 300 rpm. The bottles were illuminated from two sides by a total of four 40-W full-spectrum fluorescent bulbs (Duro-test Vitalite Lighting, Inc., Philadelphia) operated on a 16 h:8 h light:dark cycle. When on, the bulbs provided an average illuminance that totaled 4,300 lx at the two faces of each bottle (Lutron LX-101 m), which is equivalent to about 12 W/m^2 of photosynthetically active radiation (Li-Cor 2008).

To provide gas exchange, each bottle was sparged with either air or a CO_2-air mixture through a cylindrical polypropylene diffuser 36 mm long and 9 mm in diameter. Gas was delivered to the diffusers through a manifold of 4-mm inside diameter, clear vinyl tubing with manual flow control valves. For bottles sparged with air-CO_2 mixtures, gases were mixed by a gas mixer (Model 665, Matheson Co. Inc., USA) that was connected to a 50-lb tank of 99.97% CO_2 and a 2.5-psi aquarium air pump (Maxima Air Pump Model A-805, Hagen Corp., Mansfield, Mass.). The CO_2 concentration in the blend was set to maintain pH between 7.0 and 8.0. For the Roux bottles that were sparged with air alone, the 4-mm tubing was connected to a 3-W aquarium air pump (Profile 1500, Meiko Pet Corp., Taiwan).

The culture volume in each Roux bottle was 800 mL. Wastewater was introduced into the bottles with a daily draw-fill procedure at the end of the light period. Three daily hydraulic loading rates were tested—200, 267, and 400 mL of primary effluent— in order to achieve 4-, 3-, and 2-day HRTs, respectively. For the air-CO_2 sparged treatments, each HRT was run in duplicate. For the air-only treatment, the 3-day HRT was run in duplicate. Culture media temperature ranged from 23 to 25°C and did not vary between the bottles more than 1.5°C.

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Dairy Wastewater Experimental Procedures

For the dairy wastewater, algae were cultured outdoors in six 40-L rectangular glass aquarium tanks (Fig. 2). The tanks were filled with 20 L of effluent from the anaerobic digester. To better simulate light conditions in ponds, sunlight was allowed to enter the tanks only through the top water surface by masking the tank walls up to the waterline with black tape. A Plexiglas cover excluded rainfall, but a gap was provided between the cover and the tanks for ventilation.

In preliminary experiments with undiluted dairy wastewater algal growth was poor, presumably due to the high opacity of the wastewater. Therefore, the subsequent experiments reported here used 10 and 25% wastewater diluted with tap water. Each experimental treatment was run in triplicate with air sparging at 1.5 L/min for mixing and separate, simultaneous pure CO_2 sparging at approximately 0.015 L/min, which controlled culture pH.

The experiment was run during March 2007 when the average daily solar radiation was 203 W/m^2 (California Irrigation Management Information System Station #52). The average water temperature, measured daily at 3:00 p.m., was 30.6°C. Water samples were collected between 3:00 and 3:30 p.m.

Inoculation

Algae inoculum was collected from local ponds treating municipal or winery wastewater and from a creek. The inoculum samples contained a wide-ranging mixture of green algae and diatoms, which were identified by cell morphology using phase-contrast microscopy with reference to Presscott et al. (1978). Prominent genera included *Actinastrum*, *Scenedesmus*, *Chlorella*, *Spirogyra*, *Nitzschia*, *Micractinium*, *Golenkinia*, *Chlorococcum*, *Closterium*, *Euglena*, and two unidentified species. The municipal culture inoculum contained 625 mg/L volatile suspended solids (VSS) and was added to the wastewater media in a 2% (v/v) ratio. For the dairy cultures, the inoculum concentration was 500 mg/L VSS, added at a 10% (v/v) ratio.

Water Quality Analyses and Lipid Extraction

VSS concentrations were determined gravimetrically according to Standard Methods (APHA 2005). Temperature and pH were monitored to characterize growth conditions. Nutrient removal was evaluated by analyzing for nitrite, nitrate, and orthophosphate using a Dionex DX 120 ion chromatograph with an AG9-HC IonPac Guard Column, AS9-HC 4-mm IonPac IC column, DS4-1 Detection Stabilizer, and an AS40 Automated Sampler. Total ammonia nitrogen ($NH_3+NH_4^+-N$) concentrations were determined using the Ammonia-Selective Electrode Method (APHA 4500-NH_3D). Organic nitrogen was determined using the Macro-Kjeldahl method (APHA Method 4500-N_{org}).

To complete a nitrogen balance for the Roux bottle experiments, it was necessary to quantify the volatilization of ammonia. This quantity was determined by passing the sparged gas through a boric acid solution. This procedure was conducted for one Roux bottle of each duplicate. A two-hole stopper with 4-mm tubing allowed sparging gas in and directed sparged gas out and into the boric acid solution through a polypropylene diffuser. The diffuser was submersed 12 cm in a graduated cylinder under 100–200 mL of boric acid indicating solution (APHA 4500-NH_3C. 3.b.). At the end of a mass balance period, deionized (DI) water was added to the graduated cylinder to compensate for evaporation. This solution was then titrated back to its original pH, and its ammonia concentration was calculated according to APHA 4500-NH_3C.

Table 1. Initial Wastewater Characteristics

	Dairy wastewater		Municipal wastewater
Wastewater characteristics	25% dilution	10% dilution	No dilution
TSS (mg/L)	283	135	93
VSS (mg/L)	220	120	58
pH	7.9	7.7	7.2
Ammonium as N (mg/L)	30.5	16.3	39
Nitrate as N (mg/L)	<0.01	0.05	<0.01
Nitrite as N (mg/L)	<0.01	0.04	<0.01
Organic nitrogen (mg/L)	50.7	20.2	12
TKN (mg/L)	81.0	36.5	51
Total nitrogen (mg/L)	81.0	36.6	51
Phosphate as P (mg/L)	2.6	1.8	2.1

The lipid content of the VSS was analyzed gravimetrically by a procedure adapted from Bligh and Dyer (1959) by Benemann and Tillett (1987). The method consisted of solvent-based extraction to isolate both polar and nonpolar lipids from cell biomass and water. The VSS of each sample was measured to determine the concentration of algal biomass in the wastewater effluent. A 200-mL aliquot of the same sample was centrifuged in a PTFE tube to form an algae pellet for lipid extraction. After decanting, the pellet was resuspended in 4 mL of DI water and frozen until extraction. For extraction, the samples were thawed, and 5 mL of chloroform and 10 mL of methanol were added. The samples were then sonicated continuously in the centrifuge tube for 1 min. (Branson Sonifier 250 with a Model #102 tip). The samples were then placed on a shaker table overnight. The next day an additional 5 mL of chloroform and 5 mL of DI water were added to make the final ratio of chloroform:methanol:water 10:10:9. The samples were then vortexed for 30 s. After the samples had been homogenized, they were centrifuged at 7,000 rpm for 4 min. The lipids were soluble in the chloroform, which formed a dense layer at the bottom of the centrifuge tube. The remaining cell debris created a middle layer, while the methanol and water created a top layer. The lipid-chloroform layer was removed with a pipette and filtered through a 0.2-μm nylon syringe filter. The filtrate was deposited into a tared aluminum tray. The tray was then placed into a desiccator flushed with nitrogen to allow the chloroform to evaporate. A second extraction was performed by adding an additional 10 mL of chloroform to the centrifuge tube, and the mixture was again vortexed and centrifuged. This second extraction was placed into a separate tared tray and evaporated under nitrogen. The trays were then dried at 105°C for 1 h. After cooling in a desiccator, the trays were weighed to the nearest 0.01 mg. Adding the weights of the two extractions from each sample gave the total lipid weight.

Results and Discussion

Influent Wastewater Characteristics

The municipal primary wastewater characteristics, as well as the initial conditions in the dairy wastewater bioreactors immediately after inoculation are reported in Table 1. After dilution, the 25%

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Table 2. Culture Conditions in Dairy Wastewater Experiment

Day	Insolation (W/m^2)	Air temperature (°C) Max	Min	Average	Water temperature at 3 p.m. (°C)	Average pH[a] 10% dairy wastewater	25% dairy wastewater
0					32	7.7	7.9
1	50	13.6	9.9	11.4	15	7.4	7.5
2	191	17.3	7.3	11.3	30	7.2	7.1
3	228	24	11.4	16.3	36	8.9	7.4
4	228	20.9	7.5	13.1	35	9.3	7.6
5	70	14.4	9.5	12	17	7.0	7.3
6	212	17.9	10.6	13.1	32	6.5	7.1
7	169	17.8	8.6	11.6	29	7.3	8.4
8	226	15.8	4.5	9.1	27	8.6	9.5
9	246	16.3	4.5	10.4	32	7.5	7.7
10	252	22.3	4.5	12.9	37	6.4	6.4
11	247	24.2	5.1	13	34	7.1	7.3
12	246	20.9	5	11.4	36	8.3	8.0
13	242	22.1	7.9	13.4	37		7.3
14	239	20.8	7.4	13.2			
15	246	24.1	7.8	13.5			

[a]Standard deviation of replicates ranged from 0.0 to 0.5.

dairy wastewater had ammonium and orthophosphate concentrations similar to the undiluted municipal wastewater.

Culture Conditions

The laboratory municipal wastewater cultures were grown under steady conditions as described in the Methods section. However, the outdoor dairy wastewater cultures experienced widely varying conditions both daily and over the course of the experiments (Table 2). The average 24 h insolation ranged from 50–252 W/m^2. Due to manual adjustment of CO_2 flow, pH ranged from 6.5–8.9. The water temperatures in all the tanks were similar and reached as high as 37°C (Table 2).

Algal and Lipid Productivity

The semicontinuous-flow experiments with municipal wastewater reached nearly steady-state biomass concentrations after 11 days of operation, although VSS was higher on the 18th day, when lipid samples were taken (Fig. 3). For the 3-day HRT cultures, sparging with CO_2 more than doubled the VSS concentration compared to sparging with air. For the treatments with CO_2 sparging, biomass production was similar for the 3- and 4-day HRTs, with steady-state VSS concentrations of 700–800 mg/L. In contrast, the steady-state VSS concentration for the CO_2-sparged 2-day HRT treatment was only 300 mg/L. The municipal wastewater cultures were dominated by algae in the *Chlorella*, *Micractinium*, and *Actinastrum* genera.

The lipid contents of the algae from the municipal wastewater experiments ranged from 4.9–11.3% of VSS by weight (Table 3). Despite the relatively low lipid contents observed, short residence times and high biomass production rates resulted in lipid productivities ranging from 9.7 mg/L/day (air-sparged) to 24 mg/L/day (CO_2-sparged 3-day HRT).

Lipid production using dairy wastewater was measured in batch experiments with two different dilutions of wastewater (10% and 25%). Biomass concentrations increased to maximum values of 500 mg/L VSS at Day 6 for the 10% dilution (Fig. 4) and 900 mg/L VSS at Day 13 for the 25% dilution (Fig. 5). The higher biomass production for the 25% dilution was likely due to the higher nutrient concentrations (Table 1). The dairy wastewater cultures were dominated by *Scenedesmus*, followed by *Micractinium*, *Chlorella*, and *Actinastrum*. These were the same genera that dominated in the municipal wastewater cultures, except that *Scenedesmus* was absent in the municipal cultures.

For both dairy wastewater dilutions, the highest lipid content

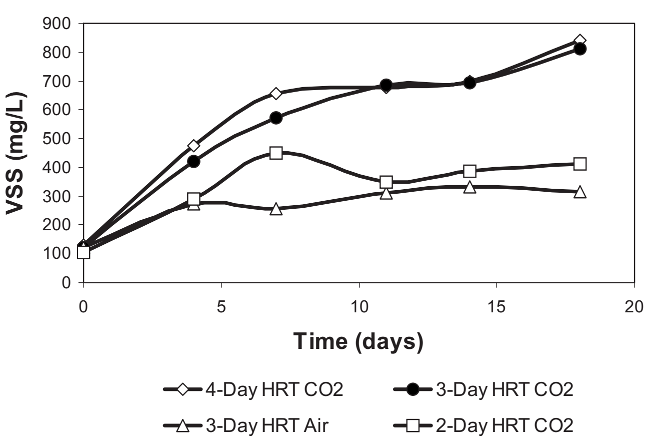

Fig. 3. Biomass concentrations during semicontinuous flow treatment of municipal wastewater (mean of duplicates)

Table 3. Lipid Productivity of Municipal Wastewater Cultures

Sample	VSS (mg/L)	Lipids (%)	Lipid content of culture medium (mg/L)	Lipid productivity (mg/L/day)
CO_2 4-day HRT	843	4.9	41.5	10.4
CO_2 3-day HRT	812	9.0	73.3	24.4
Air 3-day HRT	317	9.3	29.2	9.7
CO_2 2-day HRT	412	11.3	46.2	23.1

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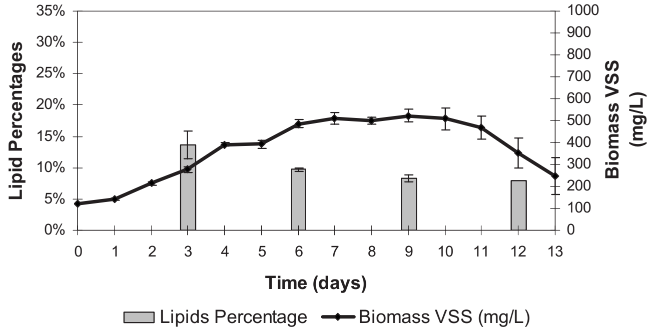

Fig. 4. Biomass concentration and cell lipid content during batch algae growth on 10% dairy wastewater (mean of triplicates)

was observed during the exponential growth phase, and it declined thereafter (Figs. 4 and 5). The total lipid content of biomass from the 10% dilution ranged from 8–14%, and that of the 25% dilution ranged from 10–29% by weight. In comparison, total lipid content of pure *Scenedesmus* and *Chlorella* cultures has been reported to range from 12–45% (Thompson 1996).

For the dairy wastewater experiments, the maximum lipid production rate was 17 mg/L/day on a volumetric basis or 2.8 $g/m^2/day$ on an area basis, achieved by Day 6 for the 25% dilution. In comparison, previous research with open-surface systems growing pure cultures have shown somewhat higher production rates ranging from 4–7.9 $g/m^2/day$ [Table 4 (Laws 1984; Thomas 1984; Brown 1990)].

In both the batch and semicontinuous experiments, peak lipid content was associated with high biomass growth rates. For the dairy wastewater experiments, the highest lipid content and productivity was achieved during exponential growth for both the 10 and 25% dilution experiments, rather than during later phases when nutrient concentrations were low (Figs. 4, 5, and 7). For the indoor municipal wastewater experiment, the highest lipid content (11%) was observed at the shortest HRT (2 days). In these semicontinuous-flow experiments, the shorter retention time corresponded to more rapid biomass growth and greater lipid productivity. Thus high lipid production was associated with rapid growth for both batch dairy and semicontinuous municipal wastewater experiments. Roessler (1990) has discussed similar results of increased lipid content in the exponential growth phase of microalgae and theorized that at lower biomass concentrations with less self-shading, algae biosynthesize lipid storage products as a means of capturing excess light energy. In contrast, others have found higher lipid content in cultures that were nutrient limited (Leman 1997; Spoehr and Milner 1949).

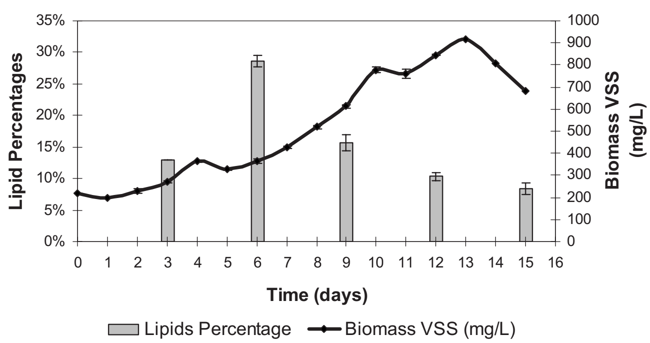

Fig. 5. Biomass concentration and cell lipid content during batch algae growth on 25% dairy wastewater

The maximum observed lipid productivity of the dairy waste reactors (2.8 $g/m^2/day$) corresponds to about 11,000 L/ha/year (1,200 gal/acre/year). Without improvements, productivity in full-scale high-rate algae ponds is expected to be lower due to factors such as winter insolation and temperature, predation, maintenance downtime, and shifts in algal strains. For example, assuming 300 days/year of operation, the productivity would be reduced to 9,000 L/ha/year (960 gal/acre/year). An additional uncertainty in scale-up estimates stems from the difference in operational modes for the dairy wastewater experiments (batch) and typical high-rate pond wastewater treatment (continuous). Theoretically, the maximum growth rate achieved in batch culture could also be achieved in continuous flow culture (Gualtieri and Barsanti 2005). Of course, the actual productivity for a full-scale system will depend on local environmental conditions, cultivation parameters, dominant algal strains, etc. Furthermore, the suitability of the algal lipids for fuel production will depend on the lipid characteristics (e.g., polarity, saturation level, and chain length) and the ease of extraction.

Much higher algal lipid productivities have been envisioned [e.g., 42,600–136,900 L/ha/year, Chisti (2007)] than were observed in this study. However, even this study's oil production estimate of 9,000 L/ha/year is 18 times greater than the 490 L/ha/year reported for soybean oil production (USDA 2005).

Nutrient Removal

For the municipal wastewater, over 99% ammonium and orthophosphate removal was achieved for CO_2-sparged treatments with both 3- and 4-day HRT (Table 5). To determine the fate of the removed ammonium and to validate the results, a nitrogen balance was calculated on four occasions over 10 days of operation. The results were similar on all 4 days, and Fig. 6 shows the balance for Day 18. The average recovery achieved was 96% with a standard deviation of 8.7%. Ammonium was the main form of nitrogen in the influent wastewater, and after algal growth, organic nitrogen was predominant (Fig. 6). Ammonia volatilization was minor, the greatest amount being <1 mg/bottle/day from the air-sparged treatment, which accounts for $<7\%$ of the influent total nitrogen. Since this treatment developed the highest pH (10.3) due to lack of CO_2 sparging, it was the most prone to

Table 4. Comparison of the Lipid Productivity of Dairy Wastewater Cultures to That Reported by Others

Study	Lipid productivity ($g/m^2/d$)	Algal species	Growth vessel	Medium
Laws (1984)	7.9	*Platymonas* sp.	Air lift flume	Sea water
Thomas (1984)	4.5	*Tetraselmis suecica*	Indoor reactor	Nutrient enriched seawater
Brown (1990)	4	*Cylcotella cryptica*	Open pond	Si deficient media
This study	2.8	Polyculture	Open reactor	Anaerobic treated dairy wastewater

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Table 5. Nutrient Removal by Municipal Wastewater Cultures

	Total ammonia nitrogen (mg/L)			Phosphate as P (mg/L)		
	Influent	Effluent[a]	% Removal	Influent	Effluent[a]	% Removal
CO_2 4-day HRT	39.0	<0.02	>99%	2.1	<0.02	>99%
CO_2 3-day HRT	39.0	<0.02	>99%	2.1	<0.02	>99%
Air 3-day HRT	39.0	6.1 (±0.89)	84%	2.1	<0.02	>99%
CO_2 2-day HRT	39.0	0.6 (±0.57)	98%	2.1	0.15 (±0.15)	93%

[a]Mean of duplicate reactors with standard deviation shown in parentheses.

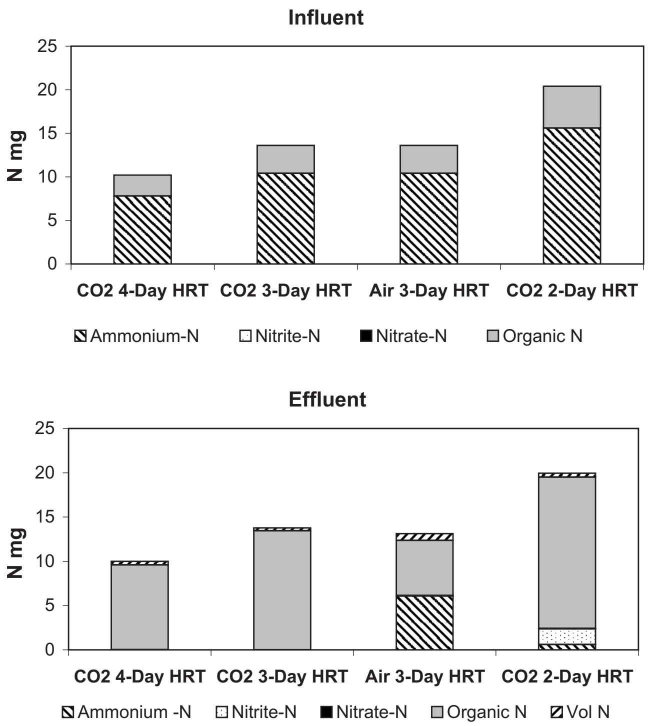

Fig. 6. Nitrogen balance for municipal wastewater cultures on Day 18 (means of duplicates). "Vol N" is volatilized nitrogen captured in a boric acid solution.

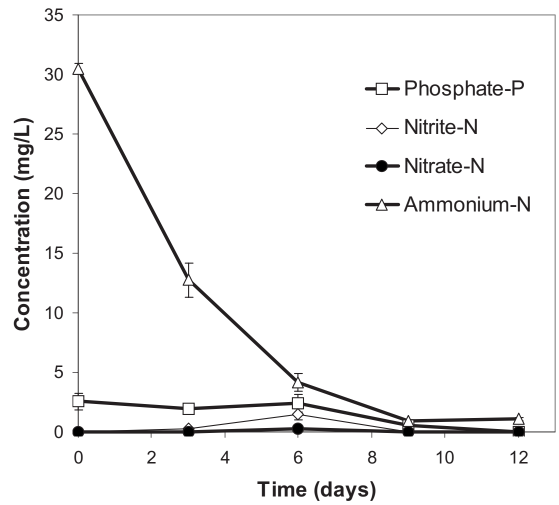

Fig. 7. Nutrient removal during batch culture (triplicates) on 25% dairy wastewater

ammonia volatilization. The nitrite observed in the 2-day HRT effluent (Fig. 6) indicates incomplete nitrification of ammonia for this short retention time.

Removal of ammonium and orthophosphate from the batch dairy wastewater was 96% and >99%, respectively by Day 15 (Table 6). For the 25% dilution experiment, initial concentrations of total ammonia nitrogen were 30 mg/L and were reduced to <5 mg/L in 6 days (Fig. 7). The initial orthophosphate phosphorus concentration of 2.6 mg/L was reduced to 0.6 mg/L in 9 days, and it was completely removed by Day 12. Nitrate concentrations were consistently below 0.3 mg/L for both conditions, and final nitrate concentrations were below the detection limit of 0.02 mg/L NO_3^-–N. Similar results were observed with the 10% dairy wastewater dilution. Nitrite showed a slight increase at Day 6 up to 0.5 mg/L NO_2^-–N, indicating some nitrification. Similar or higher ammonium removal efficiencies were observed by other researchers for algae-based treatment (Table 7).

Conclusions

This research provided a proof of concept for a wastewater treatment process that combines nutrient removal and algal lipid production for potential use as a biofuel feedstock. CO_2 supplementation was used to accelerate treatment and growth in both outdoor and indoor mixed-species cultures. Ammonium and orthophosphate removals were nearly complete for both municipal wastewater and diluted dairy wastewater. This study also contributed data on both the lipid content and the lipid productivity of wastewater-grown algae, a rarely addressed topic. Lipid content ranged from 4.9–29%, and lipid productivity reached 2.8 $g/m^2/day$. While this lipid productivity is many times higher

Table 6. Nutrient Removal in Dairy Wastewater Experiment at Day 15

	Total ammonia nitrogen (mg/L)[a]			Phosphate as P (mg/L)[a]		
	Influent	Effluent	% removal	Influent	Effluent	% removal
25% dilution	30.5 (±0.4)	1.1 (±0.1)	96%	2.6 (±0.7)	<0.02	>99%
10% dilution	16.3 (±4.8)	0.6 (±0.1)	96%	1.8 (±0.01)	<0.02	>99%

[a]Average of triplicate reactors (standard deviation, $n=3$).

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Table 7. Comparison of Total Ammonia Nitrogen Removal to That Reported for Other Algae Treatment Systems

Study	% total ammonia-N removal	Algae species	Medium
Martinez et al. (2000)	80–99	*Scenedemus obliques*	Autoclaved municipal wastewater
Lincoln et al. (1996)	99	*Arthrouspira plantensis*	Anaerobically treated dairy wastewater
Green et al. (1995)	99	Polyculture	Municipal wastewater
This study	99	Polyculture	Municipal wastewater
This study	96	Polyculture	Anaerobically treated dairy wastewater

than that of terrestrial oil plants, higher productivity is a goal of continuing research. In addition, the suitability of the lipids for fuel production by transesterification and other means needs to be determined. Overall, the waste-to-biofuel approach of this study avoids many of the cost and food competition issues of other biofuel feedstocks while providing a valuable wastewater treatment service.

Acknowledgments

Funding was provided by a U.S. Environmental Protection Agency "People, Prosperity, and the Planet" grant, as well as a U.S. Department of Energy Small Business Innovation Research grant to MicroBio Engineering, Inc. Lundquist was supported in part by the U.S. Office of Naval Research via the California Central Coast Research Partnership. The writers are grateful for consultation provided by Dr. John Benemann.

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