Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode

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A B S T R A C T

Municipal wastewater is a major source of nutrients and pollutants to freshwater and marine ecosystems and current treatment technologies are either expensive or only partially effective at removing them. We cultivated the alga Scenedesmus sp. AMDD at different pH in chemostats and batch photobioreactors in wastewater obtained from a local treatment plant. In batch mode, biomass productivities averaged 130 mg dry weight L−1 d−1, with no significant effect of growth pH detected. Maximum nitrogen and phosphorus removal rates were equivalent to 7% and 0.7% of the biomass productivity rates. Average hydraulic retention times for 90% N and P removal ranged from 6.5 to 6.65 days and 6.50–6.56 days, respectively. Recovered biomass yields ranged from 0.23 to 0.65 kg m−3 wastewater, equivalent to approximately 5–15 MJ bioenergy m−3 wastewater based on an average caloriﬁc value of 23 MJ kg−1 d.w. algal biomass. Approximately 65% of energy equivalent could potentially be recovered from the biomass through anaerobic digestion to methane. Cellular N and P content varied, with cells held longer in stationary phase showing higher C:N and C:P ratios indicative of N- and P-limitation, respectively. Analysis of trace metals in the algal biomass indicated near total depletion of Fe, Zn and Cd from the wastewater, and lower, but substantial, uptake and/or adsorption of seven other elements. Cultivation in 2 L continuous chemostats containing wastewater was also conducted. Biomass productivities in chemostats were almost 2-fold greater than the maximum rates in batch cultures. Dissolved N and P in chemostats were both either undetectable or >99% reduced compared to the wastewater. Production of bioenergy from the chemostats was estimated to be roughly 5.3–6.1 MJ m−3 d−1, significantly higher than in batch culture.

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1. Introduction

Diminishing reserves of petroleum and other fossil fuels and volatility in their market price and supply has bolstered a worldwide effort to develop renewable alternative fuels in a sustainable fashion. Microalgae have been identified for some time now as a promising feedstock for the production of such fuels, including ‘drop-in’ substitutes for petroleum-based liquid fuels for ground and air transportation [1–3]. Advantages of microalgae for this purpose include generally higher rates of areal biomass and lipid production compared to traditional agricultural crops, the potential to cultivate microalgae on land not suited for farming food crops and the demonstrated ability of microalgae to extract nutrients from what are otherwise waste streams such as municipal or agriculture wastewaters (for N and P nutrition, and trace metals) and industrial stack-gas emissions (for C nutrition) [4,5]. Nutrient concentrations in wastewater effluents can be up to three orders of magnitude higher than in receiving waters which can promote uncontrolled algal growth and subsequent oxygen starvation in the water column when these algal ‘blooms’ decompose [6]. Microalgae mediated removal of N and P from wastewater prior to its discharge has several benefits. Apart from the obvious reductions in eutrophication potential of the wastewater, the N and P are efficiently recovered and recycled into algal biomass which is suitable for the production of biofuels or non-fuel co-products such as fertilizers, in addition to the production of oxygen during photosynthetic growth [6,7,4,8]. Such ancillary benefits are not realized when traditional
chemical and mechanical systems for nutrient removal from municipal wastewaters are employed [9]. A recent life cycle analysis (LCA) study concluded that the environmental burden associated with the production of microalgae for biofuels could be considerably reduced by coupling the cultivation and processing of the biomass to wastewater treatment and industrial CO₂ capture [4]. The development of cost-effective methods for the recovery of wastewater-grown microalgae biomass will enable its use as a source of bioenergy and biofuel [5].

The application of microalgae to the treatment of municipal wastewater for pollutant remediation was first proposed almost 70 years ago [10,11]. In the decades since, commercial wastewater treatment systems have been developed in which microalgae contribute significantly to the overall treatment process with respect to biological and chemical oxygen demand (BOD and COD, respectively) and pathogen reduction, nutrient removal and water disinfection [8,12,13]. The Advanced Integrated Wastewater Pond System (AWIPS) is a treatment system consisting of a series of linked ponds in which a High-Rate Algal Pond (HRAP) receives nutrient-rich primary wastewater from an upstream oxidation pond for the photosynthetic production of microalgae [8,14]. Despite the success of AWIPS systems for wastewater treatment, the algal biomass produced in the HRAP has not traditionally been recovered for bioenergy production due to the absence of an inexpensive yet effective harvesting technology [15]. Much of the applied R+D on using microalgae for wastewater treatment has focused on the capacity of different strains of microalgae or cyanobacteria, typically members of the Chlorophyta or Cyanophyta, to grow on different sources and types of wastewaters. These studies have sought to specifically assess either biomass yields and productivities or the potential for bioenergy production, or some combination of these, but rarely all of them in the same study [16–18]. For instance, Wang et al. [19] reported robust growth of a Chlorella strain cultivated on wastewater and showed a strong correlation between nutrient load and biomass yield. Perez-Garcia et al. [17] explored the potential for algal growth mixotrophically, heterotrophically, and autotrophically on simulated wastewater. They found superior growth of immobilized Chlorella vulgaris when grown heterotrophically and improved autotrophic growth when the algal was co-immobilized with a bacterium, Azospirillum brasilense. Others have found agricultural wastewaters, particularly from swine and dairy farming operations, to be particularly appropriate for intensive microalgae cultivation. In some studies, microalgal biomass yields as high as 8.5 g L⁻¹ have been reported from swine wastewater [20]. In most cases where either municipal wastewater or agricultural wastewaters are used to grow microalgae, the extent of N and P removal can be quite high, with as much as 90% of the nutrient load reduced by the time the cultures reach stationary phase [21,18].

A nagging problem with using batch cultures of microalgae for wastewater treatment, however, is the long hydraulic retention times required for nutrient removal: 3–4 days in some studies [19,22] but more commonly 7 days or longer [21,20]. Ideally, the retention time of a given quantity of wastewater undergoing tertiary treatment by microalgae (i.e. where inorganic nutrient removal is the primary objective) should be set to keep pace with the production of the wastewater by the treatment plant. Although this is possible in theory, it is currently not practical because of the large disparity between the enormous quantity of wastewater produced at typical treatment plants and the much smaller capacity of microalgae cultivation systems to process it.

In terms of biofuel production from microalgae cultivated on municipal wastewater, Park et al. [14] have suggested that the production of methane via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product. Microalgal biomass has been shown to be a reasonably good substrate for AD with yields approaching 70% of the theoretical maxima quite common in the literature [5,23,24]. In practical terms, the integration of microalgae cultivation with wastewater treatment for methane production should be easier than for the production of other fuels since AD infrastructure is already present and operating at most large biological wastewater treatment plants. In addition to this, some of the same technologies used for dewatering waste sludges, including dissolved air flotation (DAF), for instance, can potentially be used to dewater microalgae sufficiently to feed into an AD system for energy production.

The objective of this study was to investigate the use of secondary wastewater effluent from a municipal wastewater treatment plant as a source of nutrition for intensive microalgal growth. Secondary effluent is the end result of the treatment process and typically discharged into receiving waters, unless a tertiary treatment is used to reduce eutrophying agents, although this is uncommon. There is substantial uncertainty surrounding the usefulness of secondary effluent to support high algal biomass production due to the perception that its nutritive value is low because of significant nutrient removal by the secondary treatment. We sought to reduce this uncertainty by quantifying the biomass yields and productivities that could be supported by secondary effluent in enclosed photobioreactors in batch mode, and thereby constrain the bioenergy and biomethane potential of wastewater grown microalgae. Second, we address the issue of hydraulic retention time by presenting data showing the stable and productive cultivation of microalgae in continuous chemostats at a high dilution rate (0.75 d⁻¹) for >2 weeks. We envisage that these empirical data could be used to support models and LCA analyses of microalgae based biofuel production linked to wastewater treatment.

2. Materials and methods

2.1. Microalgae strain

The chlorophytic microalgae Scenedesmus sp. AMDD was kindly provided by B. Skrupski (NRC Plant Biotechnology Institute, Saskatoon, Canada). This strain was isolated from a soil sample and identified by standard DNA sequencing of ITS1 and ITS2 regions. Axenic Scenedesmus sp. AMDD cultures were maintained in standard Bold’s basal medium [25] at 20 °C in low light. In addition to Chlorella sp. and Microactinum sp., Scenedesmus sp. are frequently isolated from active wastewater ponds suggesting that representatives of this genus are readily adaptable to wastewaters and therefore useful models for wastewater studies [15,26].

2.2. Wastewater sampling and microalgae cultivation

Secondary effluent from the Mill Cove wastewater treatment plant (Bedford, Nova Scotia) was sampled in April, May and August, 2011 from the discharge sluice immediately after UV disinfection and transported back to the laboratory for microalgae cultivation experiments (Fig. 1A). Briefly, effluent was pumped through a 1 μm string filter and 0.5 μm pleated filter arranged in series followed by passage through a 40 W UV irradiation system (Model # EU40, Aquatic Ecosystems, Apopka, FL) into a 1200 L polyethylene tank for transport. At the laboratory, the wastewater was passed through a tangential flow ultrafiltration system (500 kDa nominal cut-off size) at a flow rate of 5–6 L min⁻¹. For wastewater batch cultivation studies, microalgae were grown in proprietary Brite-Box photobioreactors [27]. These are 300 L internally illuminated PBRs enclosed by a fiberglass shell. Light was provided by ten, 32W T8 fluorescent bulbs arranged in five horizontal rows of two. Approximately 280 L of tangential flow filtered wastewater were added to each Brite-Box PBR, previously sterilized by steam treatment. Starter cultures of Scenedesmus sp. AMDD were grown in 20 L carboys in Bold’s basal medium in an environmentally controlled growth chamber at 22 °C under 100 μE m⁻² s⁻¹ light intensity. These cultures were used to inoculate the wastewater in the PBRs. The Brite-Boxes were inoculated with microalgae to an initial density of 5 × 10⁶ cells mL⁻¹. With the exception of carbon dioxide, no other nutrients were added to the cultures. Mixing was provided by aeration with a turbulent
flow of sterilized air introduced into the culture through perforations in a T-shaped air-line situated medially at the bottom of the PBR. The initial light intensity was approximately 50 μE m−2 s−1 and was increased to 150 μE m−2 s−1 then 250 μE m−2 s−1 (100% intensity) on days 3 and 4 post-inoculation, respectively. Illumination was provided continuously. The pH was set to 6.2, 6.6 or 7.0 and monitored and controlled through a pH-stat system which provided CO2 to the culture on-demand when the pH exceeded the desired setpoint. At the time of inoculation, the temperature of the wastewater was approximately 20 °C and increased slowly as the light intensity was ramped up over a period of 4–5 days. At 25 °C, a cooling mechanism was triggered whereby cold seawater was pumped through a titanium heat-exchange loop immersed in the PBR until the temperature fell below the setpoint.

Continuous cultivation of Scenedesmus sp. AMDD was conducted in secondary wastewater in 2 L water-jacketed chemostat vessels (Ace Glass, Vineland, NJ; see Fig. 1B for diagram of chemostat set-up). Wastewater from the August collection was sterilized by tangential flow-filtration and then autoclaved in place in the chemostats. Each chemostat was fitted with a pH probe pre-sterilized with ethanol and pre-calibrated with standard buffers. The pH of the chemostats was maintained at 7.0 by automated periodic injection of CO2 through a flow system for distribution to the Brite-Box PBRs. For each trial, replicate pH treatments of 6.2, 6.6 and 7.0 were established in PBRs 1 and 3, 2 and 5, 3 and 6, respectively. B. Configuration of chemostats for steady-state cultivation of Scenedesmus sp. AMDD on secondary wastewater. To establish continuous cultivation conditions, wastewater (WW) was delivered at a rate of 0.94 mL min−1 through a peristaltic pump (P) into the chemostat vessel (2L V). A separate line attached to the same pump-head actively withdrew culture and deposited it to an effluent (E) vessel for disposal. The chemostats were placed on top of a magnetic stirring plate (MS) and mixed with a stir bar. Filtered, humidified air (W) was bubbled into the chemostats. The pH was monitored continuously and maintained at 7.0 with a pH stat system (pH) by periodic injection of air enriched to 5% CO2.

For growth in continuous chemostats, volumetric productivity (BP) was calculated as the product of the biomass specific growth rate (μ) and the concentration of biomass (B) at time t according to [30]:

\[
BP \text{ (mg dwt}^{-1}\text{d}^{-1}) = \mu_t \times B_t
\]  

For growth in continuous chemostats, volumetric productivity was also estimated using Eq. (2). In this case, the specific growth rate was taken to be equivalent to the steady-state dilution rate (D, 0.75 d−1):

\[
\mu \text{ (d}^{-1}) = D \text{ (d}^{-1})
\]  

Steady-state fatty acid productivity (FAP) was calculated as the product of the BP and the fatty acid content (FA) as determined by in-situ transesterification of algal lipids followed by GC analysis (outlined below):

\[
FAP \text{ (mg dwt}^{-1}\text{d}^{-1}) = BP \times FA\%)
\]
2.4. Analysis of ammonia and phosphate in wastewater grown cultures

Aliquots of wastewater cultures in Brite-Boxes and chemostats were collected to assess the extent of microalgal-mediated N and P removal during growth. Fifteen milliliter samples were filtered through a 0.22 μm filter and stored frozen until analysis. Residual free ammonium and phosphate concentrations were determined using commercially available, spectrometry-based assay systems according to the manufacturer’s instructions using a DR 2800 portable spectrophotometer (TNT832 and TNT843; Hach Co., Loveland, CO).

2.5. Elemental analysis of biomass

Microalgae biomass samples were analyzed for carbon, nitrogen, hydrogen and sulfur (CHNS) stoichiometry using a Vario Microcube elemental analyzer (Elementar Americas, Mt. Laurel, NJ) using established techniques [31]. Freeze-dried samples (3–4 mg dry weight) were placed in tin boats and folded several times to expel any trapped air to reduce background levels of C and N contamination. Sulfanilamide standards were co-analyzed to calibrate the responses for C, H, N and S. Elemental P in microalgal biomass was determined with a commercial assay system based on the antimony phosphomolybdate method according to the manufacturer’s instructions (Phosphorus TNP-Plus, Prod # TNT844, Hach Co., Loveland, CO). Approximately 2 mg of lyophilized biomass was thoroughly resuspending with 10 mL of deionized water. A 0.5 mL aliquot of the algal suspension was transferred to the assay vial and digested for 1 h at 100 °C on a heat-block. Following cooling and addition of development reagent, the absorbance at 890 nm was measured using a DR 2800 portable spectrophotometer. For elemental P analysis from biomass samples taken from the chemostats, 5 mL of culture was sampled from the vessel and assayed directly. Controls were also run whereby 5 mL of culture was pre-filtered to remove algal cells and then digested and assayed in identical fashion to samples containing cells. Steady-state N and P assimilation rates in chemostats were estimated by multiplying the BP rates by the respective fractional N and P contents determined by elemental analysis of biomass samples:

\[ \text{N Assimilation Rate} = \frac{BP}{N_{\text{biomass}}} \]

\[ \text{P Assimilation Rate} = \frac{BP}{P_{\text{biomass}}} \]

expressed in units of mg N or P L⁻¹ d⁻¹. The net CO₂ fixation rate in the chemostats at steady-state was estimated by multiplying the BP rates by fractional C content of biomass multiplied by the mass ratio of CO₂ to C (i.e. 3.7):

\[ \text{CO₂ Fixation Rate} = \frac{BP}{C_{\text{biomass}}} \times 3.7. \]

2.6. Harvesting and dewatering

Brite-Box cultures were harvested in a continuous process centrifuge (Model Z101, CEPA Centrifuge, Lahr, Germany). For each trial, the biomass from replicate pH treatments was pooled and harvested together. The centrifuged microalgal paste (approximately 25% solids) was removed manually from the centrifuge bowl, spread evenly in plastic trays to a thickness of approximately 2 cm and lyophilized to a homogeneity of the sample, the freeze-dried biomass cake was manually removed from the centrifuge bowl, spread evenly in plastic trays to a thickness of approximately 2 cm and lyophilized to a final moisture content of approximately 3% (w/w) in an industrial freeze-drier (VirTis Ultra 35L, SP Scientific, Gardiner, NY). To ensure homogeneity of the sample, the freeze-dried biomass cake was manually mixed, blended in a commercial food processor, and stored at −20 °C until analyzed.

2.7. Biomethane potential assays from wastewater cultivated Scenedesmus sp. AMDD

 Harvested biomass samples from the pooled replicates from each pH treatment from the April Brite-Box trial were assayed for methanogenic potential in bench-top anaerobic digestion experiments. The assays were prepared based on the biochemical methane potential (BMP) test for wastewater [32,33]. In brief, a total of 10 g of fresh algal biomass was suspended in 80 mL of deoxygenated water in 500-mL serum bottles. The inoculum consisted of 20 g of granular sludge collected from a full-scale anaerobic digester and starved for 48 h prior to the assay. Two milliliters of defined media (mg L⁻¹: 2500 KH₂PO₄; 2500 NaCl; 500 CaCl₂·2H₂O; 9470 NH₄Cl; 500 MgCl₂·6H₂O; 500 (NH₄)₆MoO₄·24H₂O; 0.5 ZnSO₄·7 H₂O; 1.5 H₂BO₃; 7.5 FeCl₃·4H₂O; 50 CoCl₂·6H₂O; 0.15 MnCl₂·4H₂O; 0.15 NiCl₂·6H₂O; 0.5 AlK(SO₄)₂·12 H₂O; 0.5 nitric acid; 0.5 cyanocobalamin; 0.25 thiamin; 0.25 p-aminohippuric acid; 1.25 pyridoxine; 0.125 pantethenic acid; 0.15 resazurin; 510 2-methyl-butryic acid), 2 mL of bicarbonate buffer (mg L⁻¹ 42 NaHCO₃; 100 KHCₐO₃), and 0.5 mL of 1.25% Na₂S–cysteine solution were added to each bottle, for a final liquid volume of around 115 mL. The bottles were made anaerobic by flushing with nitrogen, sealed and incubated at 35 °C on a shaking table rotated at 150 rpm. Control bottles in which an equal volume of water was added instead of algal material were prepared and run in order to estimate endogenous methane production during the assay. Methane concentrations were determined by gas chromatography (using argon as carrier gas and thermal conductivity detection) of 0.3 mL of headspace gas sampled periodically. The assays were carried out until the methane production became negligible (~5 mL CH₄ d⁻¹). The cumulative methane yield was corrected by subtracting the endogenous methane production obtained from controls then normalized to the initial quantity of volatile algal solids (VS) and reported in units of normal m³ CH₄ kg⁻¹ VS (Nm³ CH₄ kg⁻¹ VS).

2.8. Trace element analysis

Analyses of trace metal concentrations were performed by magnetic sector inductively coupled plasma-mass spectrometry [34] with an Element 2 (Thermo Finnigan) in medium resolution using scandium and indium as internal standards. Prior to analysis aqueous samples were amended to 5% nitric acid (Optima grade); algal samples were microwave digested [35] with 100% nitric acid (trace metal grade) in closed vessels for 40 min at 200 °C. Digestion and analysis of the certified reference materials CRM-PN-A (pine needles, High Purity Standards) and SLRS-4 (river water, Natural Research Council Canada) accompanied all samples, with measured concentrations for all elements being 92–101% of their certified values. All algal samples were digested in duplicate and aqueous samples were analyzed in triplicate. The percent relative standard deviation of replicate measurements across all elements averaged 5±3% for the aqueous samples and 8±8% for digested samples.

2.9. Lipid extraction and gas chromatography analysis

For fatty acid extraction, 2–5 mg of biomass was weighed directly into tubes and 1 mL of anhydrous toluene (Sigma-Aldrich) was added, followed by 2 mL of 5% acetyl chloride (Fluka)/anhydrous methanol (99.8% Sigma-Aldrich), prepared fresh for each reaction. Tubes were purged with nitrogen, capped, mixed gently, and kept at 105 °C in a heating block for 1 h. Derivatized lipids were cooled to room temperature, washed with 5 mL of 18.2 MΩ H₂O with 5% NaCl, then 4 mL of 18.2 MΩ H₂O with 2% NaHCO₃, dried over anhydrous sodium sulfate and filtered through glass wool to exclude residual sodium sulfate. Total derivatized lipid was then dried under nitrogen, weighed, diluted to 5 mg/mL with hexane and analyzed using gas chromatography [36]. All reactions included a reagent blank to
confirm clean solvents and glassware. All samples were analyzed on an Omegawax 250 column with an Agilent 7890 gas chromatograph equipped with FID. Samples were run in constant flow mode (3 mL min\(^{-1}\)) and temperature programmed as follows: 110 °C for 1 min, then increasing by 5 °C min\(^{-1}\) to 250 °C where the program was held for 20 min (total run time 49 min). Inlet temperature was set at 250 °C, and detector temperature to 300 °C. Carrier gas was helium. Peaks were automatically integrated by Chemstation software (Agilent). A known quantity of internal standard (nonadecanoic acid, Sigma-Aldrich) was added to each tube before adding sample. The concentration of all other integrated peaks was calculated by calibrating to the area of the internal standard peak as in [37,38]. Individual fatty acids were provisionally identified by comparing retention times to two standard mixtures (Supelco 37 and PUFA No. 3, Sigma-Aldrich).

2.10. Biomass energy density analysis

Biomass energy density was determined by conducting standard heats of combustion assays using an isoperibol oxygen bomb calorimeter (model 6200, Parr Instrument Company, Moline, IL, USA) equipped with a Parr 6510 water handling system for closed-loop operation according to [39]. Benzoic acid was used as a standard. All values reported are means of three analytical replicates.

3. Results

3.1. Batch cultivation of Scenedesmus sp. AMDD in secondary wastewater effluent

The wastewater cultivation experiments described in this study were conducted in three separate trials, the first in April 2011 and the second and third in May and August, 2011. The sampled wastewater was typical of a biologically treated effluent in terms of the BOD and TSS which were both less than 20 mg L\(^{-1}\) (Table 1). Nitrogen and phosphorus concentrations varied between 20–30 mg L\(^{-1}\) and 2.2–3.5 mg L\(^{-1}\), respectively, which are also typical of secondary wastewater effluent. The calculated ratios of elemental N:P varied between 18 and 24 (mol/mol) and were similar to the canonical N:P ratio of 16 for algal biomass and, therefore, considered appropriate for the balanced growth of algae [40,41,31]. A preliminary trial was conducted in January, 2011 using secondary effluent which had only undergone primary filtration (no ultrafiltration was applied). Cultivation of Scenedesmus sp. AMDD was successfully conducted in this trial for approximately 9–10 days after which we observed a rapid and dramatic reduction in microalgal cell numbers in addition to a change in pigmentation from emerald green to pale yellowish-brown in all 6 Brite-Box PBRs (data not shown). Co-incident with these rather obvious culture ‘crashes’ was the proliferation of bacteria, flagellated protists and large ciliated, motile grazers which appeared under microscopic examination to be feeding on the cultivated algae (not shown). These observations prompted the installation of a tangential flow ultrafiltration system to more stringently filter the water to remove these contaminants prior to inoculation with microalgae. We observed significant reductions in the number of ciliated protists and bacteria (though neither was completely eliminated) in ultrafiltered wastewater in the subsequent trials through 7–11 day cultivation periods. This suggested that grazing pressure was responsible for the crashes experienced in the January trial and that introduction of a stringent tangential flow filtration step reduced contaminants sufficiently to allow batch cultivation to proceed to the point of nutrient depletion for the April, May and August trials.

From the three separate batch cultivation trials conducted in the Brite-Box photobioreactors, we evaluated the effect of different levels of CO\(_2\) supplied to the algae (and thus culture pH) on the maximum rates of growth, volumetric biomass productivity, N and P removal and the associated retention times required for 90% removal of N and P (Table 2 and Fig. 2). Lowering the pH towards the pK\(_{\text{a1}}\) of carbonic acid (~6.2) tends to promote a more rapid supply of CO\(_2\) to the cultures as less of the CO\(_2\) delivered is dehydrated to bicarbonate. There were no significant effects of CO\(_2\) on any of these parameters over the pH range investigated (pH 6.2–7.0, Table 2). For all CO\(_2\) levels investigated, the maximum specific growth rates ranged from 1.36 to 1.39 d\(^{-1}\) and the maximum biomass productivities averaged around 130 mg L\(^{-1}\) d\(^{-1}\). The calculated maximum rates of N and P removal were approximately 7% and 0.7%, respectively, of the biomass productivities for each of the CO\(_2\) treatments (Table 2). Regression analysis of the linear portion of the nutrient drawdown curves allowed estimates of the retention times required for 90% N and P removal by algal growth (HR\(_{\text{TNO3}}\), HR\(_{\text{TPrg}}\), respectively). These were estimated to be 6.55–6.65 days for N and 6.50–6.56 days for P for all CO\(_2\) levels. In each trial, P was estimated to be 90% depleted slightly ahead of N, suggesting a mild P limitation. However, these differences were not found to be statistically significant (data not shown). It is worth remarking that 90% nutrient depletion was observed at least one, three, and four days before culture harvest for the April, May and August trials, respectively, during which biomass continued to accumulate, indicating that the nutrients were taken up and stored before fixation by the microalgae. The CO\(_2\) supply during growth also had no apparent effect on the fatty acid content of the biomass at the point of harvest (Table 2). For each CO\(_2\) level, GC analysis of fatty acid methyl esters derived from extracted saponifiable lipids indicated yields of approximately 12% by weight.

The cultivation trials differed significantly in terms of the quantity of biomass recovered at the point of harvest (Table 3), but similar to what was observed with the growth and fatty acid data (Table 2), the yields from individual trials were not influenced at all by the CO\(_2\) provided during growth (Table 3). Differences in recovered yields between the 3 experiments can be explained by the point along the growth curve at which cells were harvested. Despite the reduction in contaminant load due to ultrafiltration, we observed minor initial bacterial contamination in the April trial (in all 6 PBRs), which appeared to increase up to the 6th day of cultivation. To prevent the algal cultures from crashing and to ensure that useable biomass was recovered from the trial, the cultures were pre-emptively harvested during late-log phase before stationary phase and lyophilized for further analysis. In the May and August trials, although bacteria were observed in the cultures, the degree of contamination was noticeably reduced compared to the April trial. Consequently, cultivation in these trials was allowed to continue for 10 and 11 days for the August and May trials, respectively, at which time the cultures were harvested (Fig. 2). Due to the earlier harvesting of the April trial, the recovered yield of biomass was only 40–60% of the yield from the other trials (Table 3, Fig. 2). The results from the foregoing analyses

Table 1: Characteristics of secondary municipal effluent sampled from the Mill Cove wastewater treatment plant (Bedford, Nova Scotia) on three different sampling days in 2011.

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>NH(_3)-N (mg N L(^{-1}))</th>
<th>PO(_4)-P (mg P L(^{-1}))</th>
<th>N:P Ratio (mol/mol)</th>
<th>BOD (mg L(^{-1}))</th>
<th>TSS (mg L(^{-1}))</th>
<th>Fecal coliforms (CFU 100 mL(^{-1}))</th>
<th>Plant discharge rate (m(^3) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>26/4/11</td>
<td>25.36 ± 1.15 (6)</td>
<td>2.30 ± 0.09 (6)</td>
<td>24.3</td>
<td>11</td>
<td>12</td>
<td>18</td>
<td>24,604</td>
</tr>
<tr>
<td>24/5/11</td>
<td>21.62</td>
<td>2.22</td>
<td>21.5</td>
<td>10</td>
<td>12</td>
<td>4</td>
<td>24,509</td>
</tr>
<tr>
<td>22/8/11</td>
<td>28.85 ± 0.65 (6)</td>
<td>3.51 ± 0.14 (6)</td>
<td>18.1</td>
<td>19</td>
<td>12</td>
<td>200</td>
<td>19,471</td>
</tr>
</tbody>
</table>
indicated that there were no additional gains to be made in terms of growth rate or biomass productivity by providing additional CO2 beyond that required to maintain a pH of 7.0. In the January trial, the cultures were grown at pH 6.8, 7.3 and 7.8 in paired reactors. The maximum biomass productivities at the two higher pH values were 72.1 mg L$^{-1}$ d$^{-1}$ and 66.6 mg L$^{-1}$ d$^{-1}$, respectively, which were ~40% lower than the rates in the pH 7.0 cultures (Table 2).

### 3.2. Bioenergy and biomethane valorization of municipal wastewater used for microalgae growth

The biomass energy densities determined by bomb calorimetry were remarkably consistent at roughly 23 kJ g$^{-1}$ for each CO2 treatment in all trials (Table 3). With these data, we were able to valorize a standard quantity (1 m$^{-3}$) of municipal wastewater in terms of the potential energy stored in the algal biomass cultivated in it (BMsEP). This was calculated by multiplying the yield of biomass in the wastewater at the point of harvest and the corresponding energy densities of the recovered biomass. Biomethane potential (BMtEP) assays by AD were conducted on Scenedesmus sp. AMDD biomass harvested from the April trial. Cumulative methane yields (corrected for endogenous methane production) averaged 392±5, 398±11 and 394±8 Nm3 CH4 kg$^{-1}$ dw (n=3 for each) from the

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**Table 2**

Characteristics of growth, biomass productivity, nutrient removal and fatty acid yield of Scenedesmus sp. AMDD cultivated on secondary effluent at three different conditions of pH. Growth was conducted in 300L internally illuminated Brite-Box photobioreactors. Each data point represents the pooled average from 6 independent determinations conducted in 3 separate cultivation trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth pH</th>
<th>6.2</th>
<th>6.6</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum specific growth rate ($\mu_{max}$, d$^{-1}$)</td>
<td></td>
<td>1.38 ±0.12</td>
<td>1.36 ±0.15</td>
<td>1.39 ±0.15</td>
</tr>
<tr>
<td>Maximum vol. biomass productivity (mg dw L$^{-1}$ d$^{-1}$)</td>
<td></td>
<td>132.73 ± 19.31</td>
<td>127.22 ± 24.62</td>
<td>132.42 ± 22.09</td>
</tr>
<tr>
<td>Maximum N removal rate (mg N L$^{-1}$ d$^{-1}$)</td>
<td></td>
<td>8.62 ± 0.72</td>
<td>8.19 ± 1.47</td>
<td>8.85 ± 0.91</td>
</tr>
<tr>
<td>Maximum P removal rate (mg P L$^{-1}$ d$^{-1}$)</td>
<td></td>
<td>0.93 ± 0.07</td>
<td>0.97 ± 0.09</td>
<td>0.91 ± 0.24</td>
</tr>
<tr>
<td>HRT$_{90}$ (days)</td>
<td></td>
<td>6.55 ± 0.32</td>
<td>6.65 ± 0.29</td>
<td>6.62 ± 0.30</td>
</tr>
<tr>
<td>HRT$_{90}$ (days)</td>
<td></td>
<td>6.53 ± 0.24</td>
<td>6.56 ± 0.28</td>
<td>6.50 ± 0.25</td>
</tr>
<tr>
<td>Fatty acid yield at harvest (FAMEs, % dw)</td>
<td></td>
<td>11.72 ± 1.74</td>
<td>12.08 ± 2.32</td>
<td>12.06 ± 3.05</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Growth curves of Scenedesmus sp. AMDD in secondary municipal wastewater in 300L Brite-Box PBRs from the April (A,B,C), May (D,E,F) and August (G,H,I) trials cultivated at pH 6.2 (A,D,G), 6.6 (B,E,H) and 7.0 (C,F,I). Each data point represents the mean of triplicate determinations. Growth conditions are as described in the Materials and methods.
pooled biomass samples from the pH 6.2, 6.6 and 7.0 treatments, respectively. Due to the very similar yields obtained from the samples assayed, we applied an average volumetric methane yield of 395 nm³ CH₄ kg⁻¹ dw to the biomass harvested from the May and August trials and to biomass cultivated in the chemostats. Multiplying the average methane yield by the harvested biomass yields from each trial enabled the calculation of the biomethane energy potential in 1 m⁻³ of wastewater used for algal cultivation. This value was converted to equivalent units of energy by taking as a conversion factor 0.038 MJ L⁻¹ CH₄. These data are also listed in Table 3. Calculated BMtEP was estimated to be around 3.5, 8.9 and 5.9 MJ m⁻³ MCWW for the April, May and August trials respectively. These estimates were equivalent to about 65–67% of the total potential energy of the biomass across all treatments (Table 3). We took these results to indicate that extraction of energy from municipal wastewater could be achieved with approximately 65% efficiency by conducting intensive microalgal cultivation followed by anaerobic digestion of the cultivated biomass.

3.3. Elemental composition of microalga cultivated on municipal wastewater

Analyses of the C, N and P stoichiometry of biomass harvested from batch cultures grown on municipal wastewater are presented in Table 4. The cellular C:N and C:P ratios highest, in the biomass sampled from the May trial, consistent with these cultures being held longer under nutrient depletion. The N and P quotas were highest, and C:N and C:P ratios lowest, in the biomass from the April trial which was harvested after only 7 days before stationary phase. The N content of cells harvested from the August trial was intermediate between the other trials while the P quotas were very similar to those observed from the May trial (Table 4). The N:P contents determined from analysis of the cultivated biomass from all trials were quite similar to their respective contents in wastewater (Table 1). This near complete removal indicated that the N and P levels in Mill Cove secondary wastewater are exceptionally well-balanced for algal growth.

3.4. Effect of algal growth on trace metal concentrations in municipal wastewater

The concentrations of 12 trace metals were measured in the original wastewater and in the algal biomass collected at the end of the May experiment. These data are presented in Table 5. For Fe, Cd and Zn, >88% of the metal in the wastewater was recovered at the start of the experiment could be accounted for in the algal biomass at the end of the experiment, indicating strong uptake. For Al, V and Pb approximately 70–97%, 40–50% and 30–46%, respectively, could be accounted for in the biomass. Other trace elements for which dissolved concentrations were partially reduced by algal growth were Mn, Mg, Mo, Cr and Ba.

3.5. Cultivation of Scenedesmus sp. AMDD on municipal wastewater in a continuous chemostat

The wastewater sampled for the August trial in the Brite-Boxes was also used to grow Scenedesmus sp. AMDD in 2L chemostats to assess the potential for continuous and simultaneous biomass production and nutrient removal in wastewater. These results are presented in Figs. 3 and 4 and Table 6. The chemostats were initially grown as batch cultures to the point of nutrient depletion. On day 4 post-inoculation, a peristaltic pump was switched on and fresh wastewater was introduced into the growth vessel at the same rate at which culture was removed (Figs. 1B and 3). Cell densities decreased in both chemostats by day 5 but gradually recovered to stable densities of approximately 10–12×10⁶ cells mL⁻¹. Stable cell densities and biomass concentrations were maintained for the ensuing 15 day period for which data are presented. The average biomass productivities in replicate cultures were 234±8.2 and 267±11. 6 mg dw L⁻¹ d⁻¹ for chemostats A and B, respectively, averaged from

<table>
<thead>
<tr>
<th>Growth Trial</th>
<th>Growth pH</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>August</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>6.2</td>
<td>15.73</td>
<td>2.16</td>
<td>0.24</td>
<td>8.48</td>
<td>166.43</td>
<td>19.63</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>6.6</td>
<td>15.55</td>
<td>2.24</td>
<td>0.24</td>
<td>8.09</td>
<td>156.26</td>
<td>19.31</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>7.0</td>
<td>15.58</td>
<td>2.25</td>
<td>0.26</td>
<td>8.10</td>
<td>166.37</td>
<td>20.54</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>6.2</td>
<td>15.27</td>
<td>1.17</td>
<td>0.14</td>
<td>15.19</td>
<td>288.40</td>
<td>18.98</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>6.6</td>
<td>15.09</td>
<td>1.13</td>
<td>0.14</td>
<td>15.62</td>
<td>284.33</td>
<td>18.21</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>7.0</td>
<td>15.48</td>
<td>1.18</td>
<td>0.15</td>
<td>15.33</td>
<td>264.22</td>
<td>17.24</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>6.2</td>
<td>15.04</td>
<td>1.82</td>
<td>0.24</td>
<td>9.66</td>
<td>160.27</td>
<td>16.59</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>6.6</td>
<td>14.24</td>
<td>1.86</td>
<td>0.24</td>
<td>8.91</td>
<td>155.12</td>
<td>17.40</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>7.0</td>
<td>13.33</td>
<td>1.65</td>
<td>0.19</td>
<td>9.42</td>
<td>176.28</td>
<td>18.71</td>
<td></td>
</tr>
</tbody>
</table>
this did not cause any noticeable perturbation in either the cell population or the efficiency of nutrient removal. The mean rates of steady-state CO₂ fixation were estimated by multiplying the observed C content of the biomass by the BP rates (Table 6). These averaged 416 and 474 mg CO₂ L⁻¹ d⁻¹ for chemostats A and B, respectively. Because of the continuous nature of biomass production from the chemostats it was possible to report BMsEP and BMeEP as a daily rate. Values of 5.4-6.1 MJ m⁻³ MOWW d⁻¹ for BMeEP of chemostat grown biomass are reported in Table 6 (calculated by multiplying BP by mean energy density of 23 kJ g⁻¹). The corresponding BMeEP values were approximately 65% of BMeEP and ranged between 3.5 and 4 MJ m⁻³ MOWW d⁻¹ (Table 6). Fatty acid yields determined from biomass samples taken from the chemostats were roughly 42% of those determined from batch cultures (Table 2, Table 6). Average fatty acid productivities from chemostats A and B were 11.6 and 15.7 mg FAMEs L⁻¹ d⁻¹ (Table 6).

4. Discussion

The objectives for this study were to assess the suitability of secondary municipal wastewater effluent as a growth medium for intensive cultivation of the chlorophyte *Scenedesmus* sp. AMDD. Specifically, we sought to identify the upper limit of algal productivity which could be supported in batch mode by this form of wastewater and to determine the energy value of the biomass cultivated in it, both in terms of the total energy and its associated methanogenic potential through anaerobic digestion (AD), referred to as ‘valorization’ (Table 3). Valorization ranged from 5 to 15 MJ biomass energy m⁻³

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**Table 5**

Inductively coupled plasma-mass spectrometry (ICP-MS) analysis of twelve microelements in wastewater effluent (pre-cultivation) and algal biomass from the May cultivation trial. The wastewater sample was taken after ultrafiltration before transfer into the Brite-Box PBRs. Details of the analysis are given in Materials and methods.

| Trace element | Wastewater concentration (μg L⁻¹) | Concentration in algal culture at stationary phase (assayed concentration in biomass × biomass density in culture) (μg L⁻¹) | Fractional uptake/adsorption by microalgae (%)
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6.2</td>
<td>pH 6.6</td>
<td>pH 7.0</td>
</tr>
<tr>
<td>Fe</td>
<td>95.2 ± 2.1</td>
<td>87.3</td>
<td>94.3</td>
</tr>
<tr>
<td>Cd</td>
<td>0.025 ± 0.003</td>
<td>0.025</td>
<td>0.024</td>
</tr>
<tr>
<td>Zn</td>
<td>28.5 ± 1.1</td>
<td>25.5</td>
<td>26.3</td>
</tr>
<tr>
<td>Al</td>
<td>10.2 ± 0.55</td>
<td>7.1</td>
<td>8.5</td>
</tr>
<tr>
<td>V</td>
<td>0.467 ± 0.006</td>
<td>0.188</td>
<td>0.230</td>
</tr>
<tr>
<td>Pb</td>
<td>0.166 ± 0.006</td>
<td>0.068</td>
<td>0.051</td>
</tr>
<tr>
<td>Co</td>
<td>1.88 ± 0.08</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>Mn</td>
<td>195.6 ± 5.1</td>
<td>19.2</td>
<td>24.2</td>
</tr>
<tr>
<td>Mg</td>
<td>4679 ± 183</td>
<td>680</td>
<td>668</td>
</tr>
<tr>
<td>Mo</td>
<td>1.38 ± 0.03</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Cr</td>
<td>1.57 ± 0.05</td>
<td>0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>Ba</td>
<td>13.88 ± 0.51</td>
<td>0.24</td>
<td>0.27</td>
</tr>
</tbody>
</table>

7 separate gravimetrically determined biomass measurements taken during the 15 day steady-state period (Table 6). These rates of biomass productivity were approximately 75% greater than the maximum rates obtained in batch cultures (Table 2) which, it should be pointed out, were only transient and not sustained for more than a day or so. The N and P content from chemostat cultured cells was also determined as it is for batch cultures. Direct determination of the N and P content of the biomass produced in the chemostats enabled the calculation of the steady-state rates of N and P assimilation into biomass (Table 6, using Eqs. (5) and (6)). On average, approximately 83% and 80% of the available N and P, respectively, in the wastewater were assimilated into biomass per day, assayed on 5 separate days for N and 4 for P (compare available N/P in Table 1 for August N/P assimilation rates in Table 6). Direct analysis of filtered aliquots of the chemostat cultures indicated that during steady-state, the concentrations of dissolved (≤0.22 µm) N and P were mostly below the detection limit of the assay systems, indicating their near complete removal by the algae (Fig. 4). The highest assayed concentrations of dissolved N and P in the cultures during steady-state were 7.0 µM and 2.8 µM, respectively, for chemostat A and 15.2 µM and 2.3 µM, respectively, for chemostat B, both on the 2nd day of steady-state. On day 15, the first carboy of wastewater was exhausted and replaced with a fresh one (2nd arrow). As can be seen from Figs. 3 and 4,
wastewater, depending on the yield of the culture at the point of harvest. In our experiments, up to 65% of the chemical energy stored in the algal biomass was potentially recoverable through anaerobic digestion to methane (equivalent to 3–9 MJ m⁻³ MCWW; Table 3). We also conducted continuous cultivation in chemostats using wastewater at a dilution rate of 0.75 d⁻¹ and were able to maintain steady-state conditions for more than 2 weeks (Fig. 3). Mean biomass productivities in continuous cultures were almost 2-fold higher than the maximum biomass productivities obtained in batch cultures. In addition, the high and stable biomass concentrations in the chemostats resulted in near complete N and P removal in a very short retention time (i.e. 1/0.75 d⁻¹ = 1.33 d), which was nearly 5 times shorter than in batch cultures.

The BMSep and BMETP estimates presented here represent the potential recoverable bioenergy and biomethane quantities, respectively, which could be produced by cultivating Scenedesmus sp. AMDD in secondary municipal effluent and producing methane through anaerobic digestion in a controlled laboratory setting. However, the net production of energy from microalgae grown in and recovered from wastewater would be less than this once all of the energy inputs associated with cultivation and processing of biomass were taken into account. Clearly, more favorable energy balances for microalgal fuel production will be more clearly evaluate the potential of secondary wastewater as a nutrient source for the production of algal biomass.

Secondary municipal wastewater proved to be an appropriate medium for algal growth. Stringent filtration measures were required, however, to minimize contamination by bacteria and algal grazers to permit stable cultivation for periods long enough to support significant algal biomass accumulation in batch cultures (Table 3). This requirement will pose significant and perhaps untenable challenges of cost and complexity to conducting large-scale algal cultivation on wastewater in the future. In our experiments, tangential flow ultrafiltration was used to reduce contamination sufficiently to allow algal cultivation to proceed for up to 11 days (May trial). An alternative and less costly approach to excluding undesirable bacteria, fungi, and algal grazers from large algal batch cultures might be to choose particular algal strains, especially those which naturally dominate in wastewater, which can be cultivated under stringent conditions of high pH, high ionic strength, low temperatures or other conditions that would act to limit growth of contaminating organisms [14]. Alternatively, conducting continuous cultivation in wastewater with a high dilution rate could also reduce contaminant organism viability due to their short retention time in the culture. The persistent contamination encountered in the batch cultivation trials described here is likely in part due to the long hydraulic retention times (>7 days) required for nutrient removal which may have provided sufficient time for the proliferation of algal grazers. The accumulation of organic matter from dead algal cells at stationary phase may also have contributed to sustaining the grazer population, although we did not test this specifically. We observed decreases in the numerical abundance of ciliates and other contaminants after the 11th day of steady-state cultivation in the chemostats, which was opposite to what was observed in the batch cultures where the abundance of contaminating microbes generally increased as algal biomass accumulated (data not shown). In chemostats, it appears as though the bacteria and grazers were eventually washed out of the culture.

Aside from CO2, no supplemental nutrients were added to any of the cultures used in this study. Consequently, trace element requirements had to be met by the wastewater (or from incidental contamination of cultivation equipment; see ref. [43]). The ICP-MS analysis of wastewater and recovered biomass from the May trial provided evidence for significant (40–99%) uptake and/or adsorption of the 6 trace elements Fe, Cd, Zn, Al, V, and Pb from the wastewater (Table 5). Smaller decreases (<25%) in the wastewater concentrations of an additional 6 trace elements (Co, Mn, Mg, Mo, Cr, and Ba) were documented due to the adsorption or incorporation of these elements into the algal biomass (Table 5). This is consistent with the work of other groups who have shown similar reductions in trace metal concentrations from wastewaters during algal cultivation [26,19]. Arguably, Fe and Zn are the two most important trace metals required for photosynthetic carbon fixation and both were nearly completely depleted from the wastewater. Of the two, the decrease in the iron concentration in the wastewater and its partitioning into algal biomass was greater, consistent with its relatively higher cellular quota compared to Zn [44,43]. The extent of removal was followed by Cd, Zn, Al, V, and Pb (Table 5). Although the concentration of Cd in the dissolved phase also decreased substantially, its initial concentration was very low (<0.3 ppb). The depletion in zinc can be attributed to its requirement in the enzyme carbonic anhydrase (CA), which is critical for efficient carbon fixation in all microalgae [45–47]. In some marine algae, Co and Cd can replace Zn in CA by direct replacement of the metal co-factor, in the case of Co, or by expression of Cd-requiring CA enzyme under low Zn levels [48,49]. Neither Co substitution for Zn or the Cd-CA has been shown in freshwater green algae, so their apparent uptake from wastewater by Scenedesmus sp. AMDD may be due to adsorption onto cell surface rather than active or facilitated uptake into the cell. Vanadium is often required by nitrogen-fixing soil bacteria as a co-factor in an alternative form of the enzyme nitrogenase [50]. Although not a N₂-fixing organism, the Scenedesmus strain used in the present study is a soil isolate and may therefore have a V requirement for growth. Vanadate (or Vanadate) has long been known to enhance the growth of green algae, including Scenedesmus obliquus and Chlorella pyrenoidosa [51]. In our laboratory, Scenedesmus sp. AMDD grows more rapidly when...
V-containing soil water extract is added to the growth medium (K. Park and P.J. McGinn, unpublished results). Together the trace element analyses demonstrate that the availability of trace metals in wastewater, like the major nutrients N and P, appeared to be appropriate and well-balanced for algal growth (Table 5), and algal growth resulted in reductions in the concentrations of all elements measured in wastewater.

The N and P concentrations, and their associated ratios, found in secondary wastewater are well suited to the requirements for balanced algal growth (Tables 1 and 4). Our findings differ from others who have reported N:P ratios in municipal wastewaters as high as 52:1 as low as <1:1 [19]. The yield of biomass recovered in batch experiments was highly variable from trial to trial and depended on how long biomass accumulation was allowed to proceed post-nutrient depletion (Table 3). In each of the trials, biomass continued to accumulate well beyond the point of nutrient depletion, indicating significant capacity for ‘luxury’ uptake of nutrients and storage until needed during the onset of nutrient limitation. One advantage of working with secondary wastewater over other forms is the potential to cultivate the algae into a state of N-deprivation which, in oleaginous strains, can trigger an accumulation of triacylglycerol (TAG) lipid, suitable for the production of biodiesel and other fuels [2,3]. Based on the cellular N-quotas shown in Table 4, cells from the May trial appear to have been the most N-limited, followed by the August and April trials. The same trend was seen for cellular P content (Table 4). Not surprisingly, the largest accumulation of fatty acids was detected in biomass from the May trial which averaged 15% (w/w) compared to about 11% (w/w) in the other trials (data not shown). The fatty acid yields were even lower in biomass grown in the chemostats (Table 6) indicating that this particular strain of Scenedesmus is probably not suitable for the production of hydrocarbon fuels.

In contrast to the batch cultures, a chemostat simulates the kind of continuous process required for efficient nutrient removal from scaled-up microalgal-based wastewater treatment systems. In a continuous chemostat, typically a single nutrient controls the biomass yield and therefore, the productivity of the system at steady-state [28]. Since the limiting nutrient is rapidly taken up and depleted from the inflowing media it is usually undetectable in the culture and outflow. This feature of chemostat operation is exactly the aim of any tertiary wastewater treatment strategy: to produce an effluent which is depleted in N or P, or both, to the greatest possible extent. The aim of continuous systems is to maximize biomass productivity by operating the system at the optimal combination of growth rate and biomass concentration. Despite higher yields in batch systems, their overall biomass productivity is lower than continuous systems because the time required to accumulate biomass includes the initial 2–3 days when growth rates are high but biomass concentrations are low and the final 2–3 days when the reverse is true. Related to this are the much longer hydraulic retention times required for nutrient removal in batch compared to continuous systems, as described above. In addition, chemostats allow for tighter control over the composition of the biomass than is possible with batch cultures. In future wastewater studies in our laboratory, comparisons between different modes of growth (batch vs continuous) will be conducted in the same kind of bioreactor to control for differences in growth light, temperature and mixing dynamics which were not addressed in this study.

The growth rate is an important determinant in the composition of the biomass in both batch cultures and chemostats. Algal biomass grown under a high dilution rate tends to be higher in protein and lower in lipid. Lowering the dilution rate could increase the amount of TAG produced and increase the relative amount of saturated and monounsaturated fatty acids which compose the TAGs [52,53]. For the purpose of efficient nutrient removal and consistent biomass composition, continuous cultivation systems analogous in principle to the chemostats used here, either as open raceway ponds or enclosed photobioreactors, are the only practical strategy for wastewater treatment based on microalgae photosynthesis. Further research should be conducted to evaluate the utility of these systems for wastewater nutrient remediation at larger scales over much longer time periods than tested in our exploratory experiments. Optimization of conditions for continuous cultivation of microalgae on secondary wastewater to maximize biomass productivity and bioenergy production while maintaining optimal nutrient recovery will remain an active and continuing research focus in our laboratory.

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References
