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Growth of an Indigenous Algal Consortium on Anaerobically Digested Municipal Sludge Centrate: Photobioreactor Performance and Modeling

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Abstract Centrate from dewatering anaerobically digested municipal sludge is a particular concern in wastewater treatment, as it contains high ammonia concentrations and is often recycled to the head of the plant, reducing efficiency. Algae have the potential to remove ammonia from this wastewater, while producing biomass that can be used as an energy feedstock. In this research, an indigenous algal consortium was cultivated on municipal sludge centrate in a semi-continuous photobioreactor under natural light conditions. The goals of this research were to (1) enrich an algal consortium capable of growth on sludge centrate; (2) determine the main species of the consortium;(3) measure biomass, lipid production, and nutrient removal rates; and (4) develop a simple model to describe the system. The results suggested that *Chlorella* sp. was the dominant species (95 %) in the consortium. Mean biomass productivity was 5.2 g m^{-2} day^{-1}, which was relatively high compared with other studies carried out with high ammonia strength wastewaters. Lipid production was low, comprising only 10 % of total biomass. The algal consortium effectively removed nutrients from the centrate, with observed mean removal efficiencies for total nitrogen, total phosphorus, and chemical oxygen demand of 65, 72, and 8 %, respectively. A simple irradiance-based model was developed from the fundamental Michaelis-Menten photosynthesis-irradiance (PI) response for photosynthetic organisms. A good fit to the experimental data was obtained with the irradiance-based model (R^2=0.96), indicating that the system was light limited. The results show that biomass production can be predicted based on irradiance only.

Keywords Biomass production · High-strength wastewater treatment · Indigenous algae · Natural light · Semi-continuous photobioreactor · Photosynthesis-irradiance model

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Notation

A	Reactor surface area (m^2)
a	Light attenuation constant (modified Beer-Lambert equation) (m^{-1})
b	Light attenuation constant (modified Beer-Lambert equation) (g DW m^{-3})
B	Biomass concentration (g DW m^{-3})
d_{eff}	Effective reactor depth (m)
E_k	Light saturation constant (μmol photon m^{-2} s^{-1})
I	Irradiance at a given depth (μmol photon m^{-2} s^{-1})
I_0	Incident irradiance (μmol photon m^{-2} s^{-1})
P	Net photosynthetic carbon fixation rate (μmol C m^{-2} s^{-1})
P_{m}	Maximum photosynthetic carbon fixation rate (μmol C m^{-2} s^{-1})
R_{B}	Biomass-dependent respiration rate (μmol C m^{-2} s^{-1})
R_0	Specific biomass respiration rate (μmol C g DW^{-1} s^{-1})
V	Reactor working volume (m^3)
z	Depth (m)

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Introduction

Algal biofuel production is recognized as a promising future source of renewable energy [1, 2]. Although the potential for algae-derived biofuels is high, there are many technical and economic challenges associated with algal biomass production, harvesting, and processing that must still be overcome [3]. In particular, a number of recent life cycle assessment (LCA) studies have shown that a large portion of the energy and environmental impacts associated with algal biofuel production are due to the provision of water, nutrients, and carbon dioxide needed for algae growth [2]. These impacts can be greatly reduced by using wastewater as the water and nutrient source for algae cultivation [4]. A major advantage of this approach is that the eutrophication potential of wastewater is reduced, as the macronutrients (nitrogen [N] and phosphorous [P]) present in wastewater support the growth of algae within the confines of a photobioreactor. In addition, organic matter present in wastewater favors mixotrophic metabolism (i.e., utilization of sunlight as an energy source and organic carbon for biosynthesis), which has been shown to increase biomass and lipid productivity [5]. Wastewater also contains micronutrients that support algal growth [6].

A key challenge with using raw or treated municipal wastewater for algae cultivation is that wastewater nutrient concentrations are relatively low (total nitrogen [TN] concentrations <0.04 g L^{-1}, total phosphorous [TP] concentrations <0.01 g L^{-1}). The low nutrient concentrations support low algal biomass densities, resulting in high downstream costs for thickening and dewatering [7, 8]. The use of centrate derived from municipal wastewater to support algal growth has been proposed to overcome this challenge [9]. Centrate is a waste stream produced from dewatering wastewater sludge. In particular, TN and TP concentrations present in anaerobically digested sludge centrate are the highest found in wastewater treatment plants, as anaerobic digestion mineralizes nutrients present in organic solids [4, 10, 11]. This centrate is normally recycled to the head of the wastewater treatment plant, resulting in high irregular nutrient loads that can upset mainstream treatment processes, increase energy and chemical costs, and reduce efficiency by retreating pollutants. Therefore, the treatment of anaerobically digested centrate using algae is particularly advantageous [34, 35].

Although using centrate for algae cultivation offers high growth potentials compared to other wastewater streams, approximately 60 % of the TN in centrate is present as ammonium (NH_4^+), with the other major fraction being organic nitrogen [12]. The high NH_4^+concentration is a toxicity concern, as free (unionized) ammonia (NH_3) dissipates transmembrane proton gradients in algae [13, 14]. The equilibrium shift between these two forms is highly influenced by pH, with higher concentrations of free ammonia present at higher pH. Prior studies have addressed this problem by using different measures, which are discussed later [15–17].

In this paper, the cultivation of an indigenous algal consortium using centrate derived from anaerobically digested municipal sludge was demonstrated in semi-continuous column photobioreactors under natural sunlight conditions. Biomass production was modeled using a simplified irradiance-based model developed according to Michaelis-Menten photosynthesis-irradiance kinetics. Treatment of the centrate was evaluated by measuring influent and effluent concentrations of nutrients and organics.

Materials and Methods

Indigenous Algae Collection and Photobioreactor Start-up

A filamentous, indigenous algal mat (a clump or debris of mainly filamentous algae mixed with bacteria) was harvested from a secondary clarifier at the Howard F. Curren Advanced Wastewater Treatment Facility (HFCAWTF) in Tampa, Florida. The algal mat was gently swirled in filtered centrate (described below) to suspend the indigenous microalgae present. The mixture was allowed to grow in 0.4 L of 0.2-μm filtered centrate in a 1-L flask. A 2 % CO_2-air mixture was bubbled through the flask at a flow rate of 0.5 L min^{-1}. The flask was maintained at room temperature (~22 °C) with a 16/8-h light/dark cycle under artificial light conditions of 20.1 mol m^{-2} day^{-1}. A 10-day growth period was initially allowed before transferring the suspended microalgae into a 1-L bottle containing 600 mL of filtered centrate. Serial transfers were carried out to ensure that the algal consortium was adapted to the centrate and in the late log or stationary phase of growth by incubating the suspension until the total suspended solid (TSS) concentration reached 2.0 g dry weight (DW)L^{-1} and then transferring 0.05 L of the suspended indigenous algal consortium into 0.6 L of fresh filtered centrate. The resulting algal culture was used to inoculate the pilot-scale photobioreactors.

Scale-up, Photobioreactor Setup, and Maintenance

Vertically hanging tubular plastic bag photobioreactors were obtained from the Faculty of Plant and Environmental Sciences at the Norwegian Life Sciences University (UMB), Ås, Norway. Each photobioreactor column had a height of 2.73 m, a diameter of 0.12 m, and a total volume of 10 L. Centrate was added until a total operating volume of 7.0 L was achieved. The algal culture described above was added to achieve an initial TSS concentration of 0.6 g DW L^{-1}. The photobioreactor was operated as a batch system for 2 weeks to increase the initial biomass density. Subsequently, the system was operated as a semi-continuous photobioreactor at a mean

cell residence time of 7 days (dilution factor of 0.14 day^{-1}) by removing 14 % (1 L) of the contents of each cell on a daily basis and replacing it with new centrate.

Algal growth experiments were conducted under natural illumination (discussed in detail in the "Results and Discussion" section) in a temperature controlled (25–32 °C) greenhouse at the University of South Florida Botanical Gardens in Tampa, Florida (27.9710° N, 82.4650° W) between November 1 and December 19, 2011. A CO_2 mixture 2 % CO_2/air mixture was bubbled into the culture from the bottom of each photobioreactor column using compressed gas sources to provide a carbon source and maintain the culture in a well-mixed condition. The gas flow rate was maintained at 0.5 L min^{-1} in each column using rotameters supplied with needle valves (Cole Parmer Inc., Vernon Hills, IL) and coarse bubble diffusers.

Centrate Collection, Processing, and Storage

HFCAWTF digests a mixture of municipal primary and waste activated sludge (WAS) in a mesophilic (35 °C) single-stage anaerobic digester with a 21-day SRT. Biosolids are dewatered using a gravity belt thickener, with polymer addition. The belts are periodically washed with treated wastewater effluent that may significantly dilute the centrate. Centrate was collected weekly from HFCAWTF and filtered using a filter cloth to remove large biosolids, increase light transmission, and reduce solids degradation in the feed. The % light transmissivity (at 254 nm) of the filtered centrate was measured prior to its use and was found to vary between 81 and 90 %. Total nitrogen (TN) and total phosphorous (TP) concentrations in the centrate were measured on the day of collection and adjusted to between 0.20 and 0.25 g L^{-1} TN and 2.5×10^{-2} and 7.63×10^{-2} g L^{-1} TP, by dilution with local groundwater or addition of $(NH_4)_2SO_4$ and/or KH_2PO_4. New feed was collected every 2 weeks and stored in a dark refrigerator at 4 °C.

Sampling and Analytical Methods

Photobioreactor samples were analyzed daily for TSS, dissolved oxygen (DO), dissolved CO_2, optical density at 670 nm, and pH. Influent and effluent concentrations of TN, TP, chemical oxygen demand (COD), nitrate (NO_3^--N) and NH_4^+-N were measured weekly. Changes in TSS were used as an indication of areal biomass productivity, which is reported here as gram dry weight (DW) per square meter per day. An Onset® HOBO U12 data logger was used to record irradiance, ambient temperature, culture temperature, and relative humidity every 15 min. The logged data was in units of lux (1 lx= 1.85×10^{-2} μmol photon m^{-2} s^{-1}).

Analyses were conducted according to *Standard Methods* for TS (2540G), TSS (2540B), DO (4500-O C), NO_3^--N (4500-NO_3 B), TN (4500-N), TP (4500-P C), and COD (5220 D) [20]. NH_4^+-N concentration was determined by the salicylate method using Hach test vials (Loveland, CO). The estimated method detection limits (MDLs) for TN, TP, and NH_4^+-N were (g L^{-1}): 7.0×10^{-3}, 0.06×10^{-3}, and 0.6×10^{-3}, respectively. Culture pH was measured using a calibrated pH meter and probe (Metrohm, Riverview FL or Teledyne Isco, Lincoln, NE). Lipid content was determined gravimetrically at the end of the experiment (day 47) using the method of Bligh and Dyer [19]. Chlorophyll content for the consortium was determined using a methanol extraction method described by Franco et al. [21]. Total chlorophyll was calculated using Lichtenthaler equations [22].

Algal Species Identification and Enumeration

Samples were collected at the end of the experiment (day 47) and shipped to the Environmental Biotechnology Laboratory in the Department of Soil & Water Science at the University of Florida for species identification and enumeration. Algae were microscopically observed using a Nikon Labophot (Nikon Corporation, Tokyo, Japan) after brief (10 s) centrifugation at 15,000 rpm (Eppendorf 5414, Hamburg, Germany). Each resultant cell paste was observed and keyed to genus level following Wehr and Sheath based on algal cell morphology [18]. Algal cells were counted on a Bright-Line hemacytometer with improved Neubauer ruling (American Optical Co., Buffalo, New York). Triplicate counts were made from two grab samples, and the average counts were taken. Cell numbers per milliliters were calculated [23]. Genera were counted separately and compiled for a total cell count and relative species composition. Taxonomic composition was recorded as percent relative abundance of the total population.

Algal Growth Modeling

It was assumed that the photobioreactor system is a completely mixed semi-batch reactor. An overall mass balance for the photobioreactor system yields the following:

$$\frac{dB}{dt} = r - \frac{Q}{V}B \tag{1}$$

where B is the biomass concentration (g DW m^{-3}), V is the working volume of the photobioreactor (m^3), and Q is the flow rate (m^3 s^{-1}). The average mean cell residence time can be calculated as V/Q, which was maintained at 7 days.

The modeled biomass prior to the time of harvest (B_{tp}) was calculated from the following:

$$B_{tp} = B_{t-\Delta t} + r(\Delta t) \tag{2}$$

where r is the growth rate (g DW m^{-3} s^{-1}) and Δt is the elapsed time since the last harvest (s). The biomass concentration after harvest (B_{ta}) was calculated as follows:

$$B_{ta} = B_{tp}\left(1 - \frac{V_H}{V}\right) \quad (3)$$

where V_H was the harvest volume (m^3) or the volume of the reactor contents removed each day. The model was programmed to match the semi-continuous operation of the photobioreactor, such that the predicted biomass concentration at 15:00 h (once a day) was adjusted to match the feed and harvest flow, and algal growth rate. The algal growth rate depends on both nutrient availability and irradiance. However, in this study, irradiance was considered the limiting factor for microalgae growth as nutrients were assumed to be in excess (Table 1). Since growth rate is directly related to carbon fixation rate, a simple irradiance-based model was applied in this work according to the Michaelis-Menten formulation [24], which relates light to carbon fixation:

$$P(z) = P_m \frac{I(z)}{E_k + I(z)} \quad (4)$$

where $P(z)$ is the gross carbon photosynthetic rate (μmol C m^{-2} s^{-1}), P_m is the maximum photosynthetic rate (μmol C m^{-2} s^{-1}), $I(z)$ is the irradiance (μmol photon m^{-2} s^{-1}) at depth z (m), and E_k is the light half saturation constant (μmol photon m^{-2} s^{-1}); that is, the irradiance value at which the photosynthetic rate is half of the maximum value. Because the system is uniformly mixed, algal cells move in and out of the light field. Hence, a time-averaged light is experienced, which is less than the incident light. The propagation of light through the culture can be defined according to a modified Beer-Lambert relationship as follows [24]:

$$I(z) = I_0 \exp\left(-\frac{aBz}{b + B}\right) \quad (5)$$

where I_0 is incident irradiance (μmol photon m^{-2} s^{-1}), a (m^{-1}) and b (g m^{-3}) are attenuation constants, and z (m) is the cross-sectional light path [24]. In this study, values for a and b were obtained from Yun and Park [24] and are shown in Table 2. By integrating through the effective light path, d_{eff} (m), the net photosynthetic rate per unit surface area, P_{net} (μmol C m^{-2} s^{-1}), is given by

Table 1 Mean nutrient values for influent and effluent

Parameter	Influent (g L^{-1})	Effluent (g L^{-1})
Mean TN concentration	0.22	0.08
Mean NH_4^+-N concentration	0.22	0.05
Mean TP concentration	0.03	0.01
Mean COD concentration	0.13	0.11

Table 2 Model parameters

a	1,041 m^{-1}	Yun and Park [24]
b	1.03 g DW m^{-3}	Yun and Park [24]
d_{eff}	0.12 m	Measured
E_k	73.1 μmol photon m^{-2} s^{-1}	Calibrated
P_m	5.53 μmol C m^{-2} s^{-1}	Calibrated
R_0	0.15 μmol C g DW^{-1} s^{-1}	Calibrated

$$P_{net} = P_m\left(\frac{b + B}{aB}\right)\ln\left(\frac{I_0 + E_k}{E_k + I_0 \exp\left(-\frac{aBd_{eff}}{b + B}\right)}\right) - R_B \quad (6)$$

where R_B is the biomass-dependent respiration rate (μmol C m^{-2} s^{-1}) and was obtained by

$$R_B = \frac{R_0 BV}{A} \quad (7)$$

where A is the illuminated surface area (m^2), and the specific biomass respiration rate, R_0 (μmol C g DW^{-1} s^{-1}), was obtained by fitting the data.

The algae growth rate, r, needed for Eq. 1, was calculated from P_{net} (Eq. 6) from

$$r = \frac{24(10)^{-6}}{d_{eff}} P_{net} \quad (8)$$

The effective path length of the photobioreactor (d_{eff}) was calculated as the working volume divided by the illuminated surface area (d_{eff}=V/A). In Eq. 8, the numerator was obtained by assuming that the DW of algae consists of 50 % carbon (numerator=12 g C mol^{-1}×2 g DW biomass g C^{-1}×10^{-6} μmol mol^{-1}).

Results and Discussion

Microscopic Identification and Enumeration of Algae

Identifying and enumerating the indigenous species in the algal consortium is important to determine their relative contribution to biomass and lipid content and provide greater understanding of ecological relationships. The primary genera identified within the photobioreactor samples resembled *Chlorella*, *Chlamydomonas*, and *Stichococcus*, which comprised 95.2, 3.1, and 1.1 % of the total cell population, respectively (Fig. 1). Several other species of algae were rarely observed and included the following: *Scenedesmus*, *Trachelomonas*, and unidentified diatoms. These genera, along with unidentified algae, comprised ~0.6 % of the total

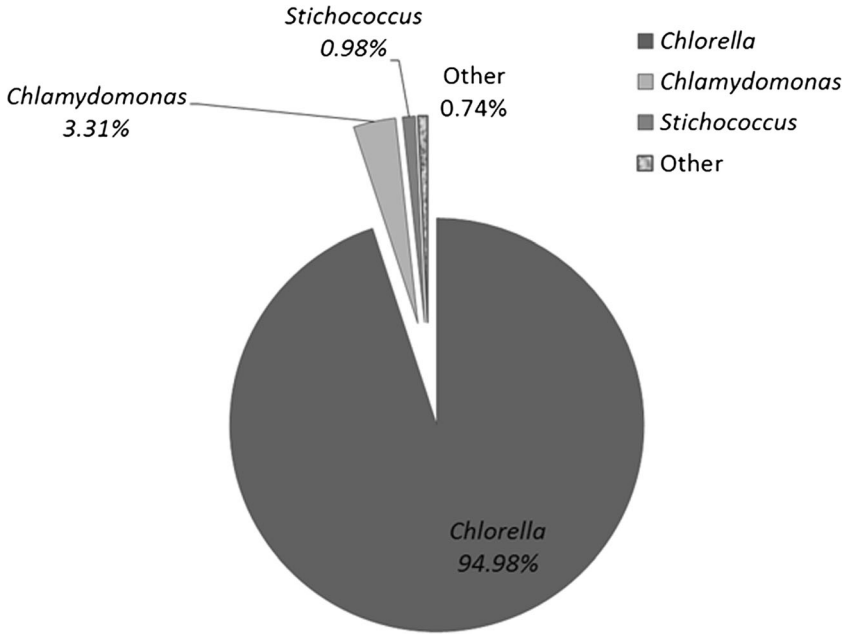

Fig. 1 Composition of indigenous algal consortium

algae population. Rotifers were also observed but were not identified or counted. An image taken of a view under the light microscope of the algal community is shown in Fig. 2. Most of the cells were spherical, which is typical for *Chlorella*.

Lighting Conditions

Light is one of the necessary ingredients supporting the metabolism of photoautotrophs. Most (45 %) of the visible light spectrum between 400 and 700 nm is available for algal growth [25]. Approximately 8.5 MJ is required to produce 1 mol of glucose [16]. The amount of instantaneous photosythetically active radiance (PAR) and total daily insolation varied over the cultivation period from November through December 2011. Incident irradiance was, on average, low given the time of the year. The maximum instantaneous PAR was 566 μmol photon m^{-2} s^{-1} (Fig. 3). The mean insolation over the period was 6.1±1.5 mol photons m^{-2} day^{-1}. The maximum and minimum insolation was 9.4 and 2.3 mol photons m^{-2} day^{-1}, respectively (Fig. 4). Cultivation in the greenhouse reduced outdoor PAR by 60–70 %. However, since the photosynthetic rate saturates at high irradiance, significant biomass productivity was still observed (Figs. 5 and 6). It was clear that algal growth was light dependent, as on low insolation days (days 20 and 27), low biomass accumulation was also observed. It appears that through constant dilution, a continuous production process can be achieved that effectively utilizes the available PAR.

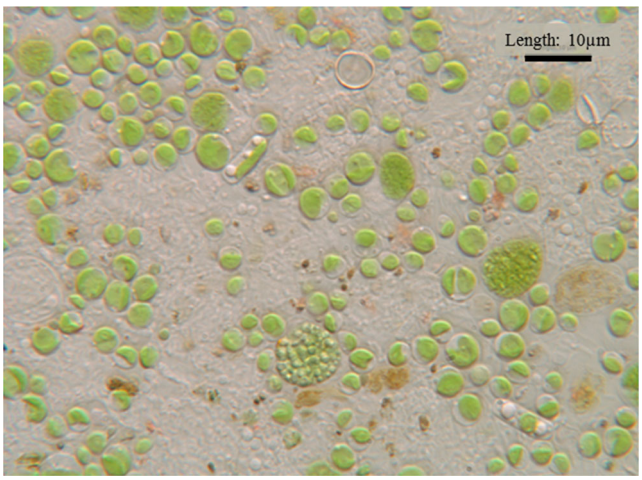

Fig. 2 Light microscope image under ×1,250 magnification

Algal Biomass Growth

The indigenous algal consortium was able to grow and survive on the wastewater centrate under semi-continuous

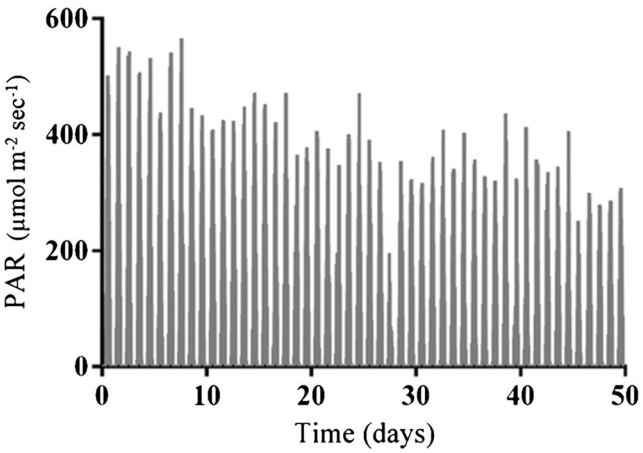

Fig. 3 Instantaneous PAR (μmol m^{-2} s^{-1}) over the experiment

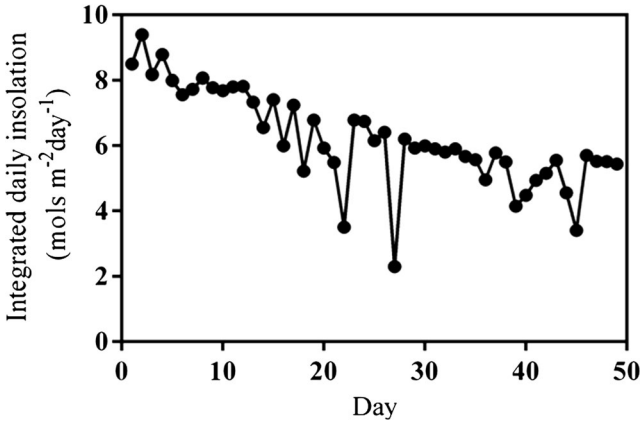

Fig. 4 Integrated daily insolation (mol m^{-2} day^{-1})

photobioreactor conditions. In this paper, the standing biomass (g DW m^{-2}) refers to the total mass of algae in the photobioreactor normalized by the illuminated surface area. Harvested biomass (g DW m^{-2}) refers to the normalized biomass collected daily from the photobioreactor. The sum of the standing and harvested biomass was used to calculate the cumulative or total biomass over time (g DW m^{-2}). The maximum standing biomass achieved was 84 g DW m^{-2} (Fig. 5). Final cumulative biomass at the end of the growth period was 299 g DW m^{-2} (Fig. 6). Although there was significant variability in the observed standing biomass, a pseudo-steady state was observed, where the measured standing biomass ranged between 30 and 90 g DW m^{-2}. It is suspected that the variability could be attributed to periodic settling of biomass as a result of cell flocculation. Flocculation could be associated with growth of bacteria in the system and daily variations in medium pH [26, 27].

Biomass Production Modeling

Comparisons of the measured and predicted standing and cumulative biomass are shown in Figs. 5 and 6, respectively. The model captures the increase in standing biomass over the first 2 weeks of cultivation (Fig. 5). Thereafter, the model predicts a pseudo-steady state in the standing biomass.

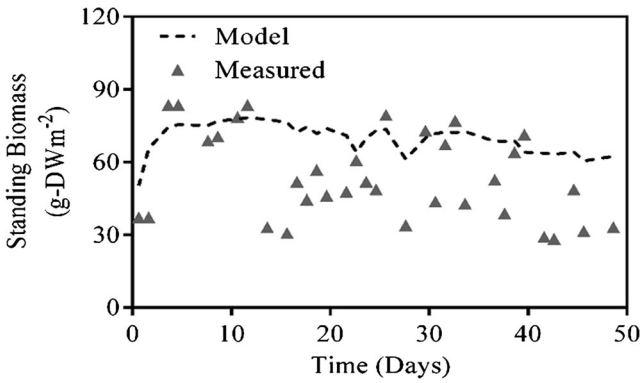

Fig. 5 Standing biomass over the duration of the experiment

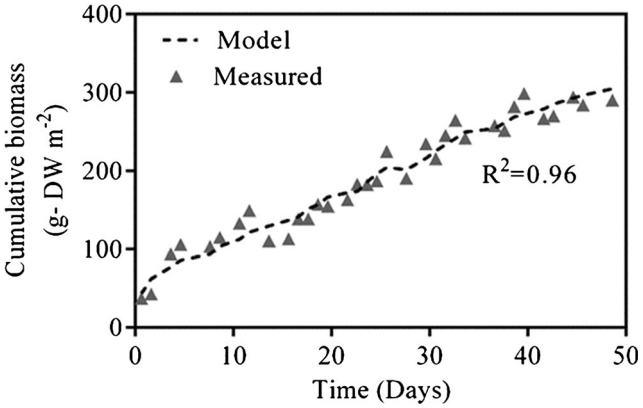

Fig. 6 Cumulative biomass over the duration of the experiment

However, as previously discussed, the measurement of biomass varies significantly between 30 and 90 g DW m^{-2}, likely due to periodic settling and resuspension of cells. An excellent fit of the model to the cumulative biomass data was achieved (R^2=0.96).

Values of E_k and P_m were obtained using a nonlinear least square fitting procedure and are shown in Table 2. The observed E_k and P_m values are similar to those reported by other authors for *Chlorella* [24]. The results demonstrate that the simple irradiance-based model applied here was sufficient to describe the photobioreactor system, indicating that biomass productivity was mainly light limited. The simplicity of the approach lends itself to ease of application for industrial prediction of biomass under similar conditions or a determination of how irradiance will influence biomass productivity.

Lipid Production

Lipid analyses conducted at the end of the experiment showed that lipids accounted for 10 % of the total dry biomass. Due to the low lipid content, evaluation of the contribution of bacteria and other organic particles to the total lipid content was not carried out. The lipid productivity may have increased if the mean cell residence time was increased, which would result in decreased photobioreactor nutrient concentrations [15]. Prior studies have shown an inverse relationship between lipid production and TN concentration [5]. Therefore, it is not surprising that lipid content was low for algae grown on high TN strength wastewater. Lipid content greater than 30 % is generally required for biodiesel production to be economically viable [2]. However, alternative forms of fuel production can include methane production via anaerobic digestion [9] or hydrothermal liquefaction of algal biomass for fuel production [33].

Nutrient and COD Removal

Mean removal efficiencies for NH_4^+, TN, and TP were 74.2, 65.0, and 72.6 %, respectively, as shown in Fig. 7. The TN

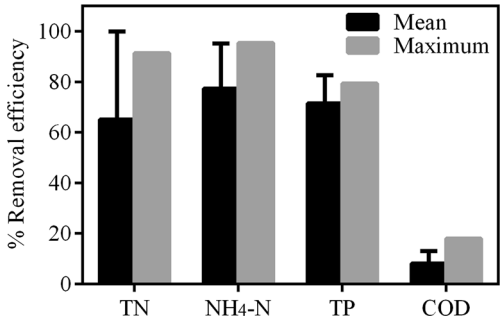

Fig. 7 Removal efficiency of nutrients and COD

removal efficiency (91.4 %) and maximum TN removal rate (0.03 g L^{-1} day^{-1}) were high, especially considering that the mean cell residence time was half that of similar studies (Table 2). The main nitrogen removal mechanism was most likely cell synthesis. Based on a simple mass balance on nitrogen, the mean biomass accumulation rate of 0.02 g/L was found to be reasonably commensurate with the average measured influent and effluent nitrogen concentrations. This assumes some uncertainty in the true N content of algal cells. Very little nitrogen removal could be attributed to NH_3 stripping or denitrification. The maximum photobioreactor pH was 7.32, and free NH_3 would have accounted for only 1 % of the total ammonia nitrogen at this pH [28]. Denitrification was an unlikely mechanism since the system was fully aerobic.

Nitrogen and phosphorous are the macronutrients required in the largest amount to support algal growth. The ratio, quantities, and forms of N and P vary widely in different types of waste streams and at different points within wastewater treatment plants [6]. The N/P ratio required for optimal algal growth is between 6.8 and 10 g/g [10]. Although an N/P ratio of 7.2 g/g can be calculated from an assumed algal biomass molecular formula of $C_{106}H_{263}O_{110}N_{16}P$, the actual N/P ratio required is dependent on the form of the nutrients supplied (e.g., NH_4^+, NO_3^-, and organic N) and their bioavailability [29]. In this study, the average N/P ratio in the centrate was maintained at 6.3, which is slightly below the optimal N/P ratio, indicating that nitrogen limited growth.

The COD removal efficiency observed in this study was relatively low (8 %). *Chlorella* spp. are capable of mixotrophic metabolism; however, in this study, they mainly utilized inorganic carbon from the carbon dioxide provided. This was most likely due to the low bioavailability of organic carbon in centrate from anaerobic digesters, as most of the easily degradable organics are converted to biogas (a mixture of methane and carbon dioxide) during the anaerobic digestion process [9].

Comparison with Other Studies

A summary of recent studies that investigated the growth of algae on centrate is shown in Table 3. The mean algal productivity achieved in this study (5.2 g DW m^{-2} day^{-1}) was higher than that in many of these studies. As discussed previously, the high concentrations of NH_4^+ typical of anaerobically digested sludge centrate poses a potential toxicity problem for algae cultivation, as concentrations greater than 0.2 g NH_4^+-N L^{-1} have been shown to significantly inhibit algal productivity [30]. Operational measures that can be used to reduce ammonia inhibition include the following: (1) combining different waste streams to reduce ammonia concentrations, (2) using indigenous algae species, and/or (3) using a semi-continuous or continuous mode to dilute ammonia concentrations. Cabanelas et al. [10] and Travieso et al. [15] combined waste streams. Cabanelas et al. [10] compared algal growth on 13 different waste streams, including centrates with five different N/P ratios (0.7–15.0) and determined that algal productivity was higher with centrate with an N/P ratio of 2.0 than with all other waste stream sources [10]. Travieso et al. [15] used *Chlorella vulgaris* to treat a combination of settled swine waste (with NH_4^+-N concentrations of 0.34 g L^{-1}) and raw municipal wastewater in a 1:60 volume ratio.

Using adapted indigenous algae may be particularly advantageous to overcome the ammonia toxicity problem, while achieving a high level of wastewater treatment for nutrients and organics. High algal growth and nutrient removal rates have been achieved with indigenous algae acclimated to high NH_4^+ concentrations, such as livestock waste [15, 30], dairy waste [6, 31], and centrate from municipal wastewater [11, 4]. Growth rates of 14 strains of indigenous microalgae on centrate were examined by Li et al. [4]. *Chlorella kessleri* and *Chlorella protothecoides*, which were capable of mixotrophic metabolism, had the highest net growth rates.

The photobioreactor system used in this study was operated in semi-continuous mode by removing 14 % of the total reactor volume each day and replacing it with fresh centrate. This allowed NH_4^+-N concentrations in the photobioreactors to be maintained at a relatively low level through dilution, while providing enough residence time in the photobioreactor for algal growth and nutrient metabolism. This dilution approach has been used in prior studies to reduce the exposure of algae to toxic levels of NH_4^+-N found in sludge centrate [15–17, 31].

Conclusions

A photobioreactor operated under semi-continuous conditions with an indigenous algae consortium was successful at production of algal biomass, while reducing high nutrient levels in wastewater centrate. The consortium, which was harvested from the wastewater clarifier, consisted of more than 95 % of a species resembling *Chlorella* sp. The application of a simple irradiance-based model was sufficient to describe biomass

Table 3 Summary comparing results obtained in this and previous studies

Feed used	Reactor operating conditions	Algae species used	Mean TN feed concentration(g L^{-1})	Light period (hr) and insolation (mol m^{-2} day^{-1})	Max. productivity (g m^{-2} day^{-1})	Max. TN removal (%)	Lipid content (%)	Reference
Centrate from the activated sludge process	Batch for 7 days, then continuous for 7 days (total=14 days)	*Chlorella*	0.15	14/10 13.0	I[a]	89.1	NP	Cabanelas et al. [10]
Raw and autoclaved centrate from the activated sludge process	Batch, 14 days	*Chlorella*	0.12–0.13	24/0 4.3	13.0	89.0	11.0[c]	Li et al. [11]
Anaerobically digested municipal centrate	Batch, 12 days	*Chlorella*	0.2–0.4	12/12 5.2	6.8	91.0	NP	Yuan et al. [12]
Mixture of settled swine waste and sewage	Continuous with 4–14-day HRT	*Chlorella vulgaris*	0.02	Natural lighting 46.8–61.6	38.2	26.1	NP	Travieso et al. [15]
Anaerobically digested swine centrate	Semi-continuous	*Scenedesmus*	1.22	12/12 8.6	I[a]	89.0	NP	Park et al. [16]
Anaerobically digested municipal centrate	Semi-continuous, 12 days	*Chlorella*	0.62	Natural lighting, NP	13.0	98.9	NP	Rusten and Sahu [17]
Anaerobically digested dairy centrate	Semi-continuous, 7-day HRT	*Microspora willeana*	0.33	16/8 3.5–12.1	5.5	39	NP	Wilkie and Mulbry [31]
Centrate from the activated sludge process	Batch, 12 days	*Auxenochlorella protothecoides*	0.17±0.038	24/0 5.2	I[a]	73.6	20.8	Hu et al. [32]
Anaerobically digested municipal centrate	Semi-continuous, 7-day HRT	Mixed consortia (*Chlorella* is dominant)	0.20–.25	Natural lighting 2.3–9.4	5.2[b]	91.4	10.0	This study

This may be slightly less than total lipid content

NP not provided

[a] I=insufficient information provided to calculate aerial productivity

[b] Mean

[c] FAME lipid %

development in the photobioreactor, including cumulative and standing biomass. While maximum TN removal rates were high compared with prior studies, low COD utilization may have been due to the low bioavailability of COD in the centrate. The consortium had low lipid content, indicating that it should be used as feedstock for anaerobic digestion.

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