Uptake of Inorganic and Organic Nutrient Species During Cultivation of a *Chlorella* Isolate in Anaerobically Digested Dairy Waste

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A natural assemblage of microalgae from a facultative lagoon system treating municipal wastewater was enriched for growth in the effluents of an anaerobic digester processing dairy waste. A green microalga with close resemblance to Chlorella *sp. was found to be dominant after multiple cycles of sub-culturing. Subsequently, the strain (designated as LLAI) was isolated and cultivated in 20× diluted digester effluents under various incident light intensities (255–1,100 μmoles m^{-2} s^{-1}) to systematically assess growth and nutrient utilization. Our results showed that LLAI production increased with increasing incident light and a maximum productivity of 0.34 g L^{-1} d^{-1} was attained when the incident irradiance was 1,100 μmoles m^{-2} s^{-1}. Lack of growth in the absence of light indicated that the cultures did not grow heterotrophically on the organic compounds present in the medium. However, the cultures were able to uptake organic N and P under phototrophic conditions and our calculations suggest that the carbon associated with these organic nutrients contributed significantly to the production of biomass. Overall, under high light conditions, LLAI cultures utilized half of the soluble organic nitrogen and >90% of the ammonium, orthophosphate, and dissolved organic phosphorus present in the diluted waste. Strain LLAI was also found to accumulate triacylglycerides (TAG) even before the onset of nutrient limitation and a lipid productivity of 37 mg-TAG L^{-1} d^{-1} was measured in cultures incubated at an incident irradiance of 1,100 μmoles m^{-2} s^{-1}. The results of this study suggest that microalgae isolates from natural environments are well-suited for nutrient remediation and biomass production from wastewater containing diverse inorganic and organic nutrient species.* © 2016 American Institute of Chemical Engineers *Biotechnol. Prog.*, 32:1336–1342, 2016
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Introduction

Production costs for algal feedstocks can be reduced through co-location of cultivation ponds/photobioreactors with preexisting large-scale processes to allow for better materials and energy integration.[1–3] Cultivation of microalgae using effluents of anaerobic digesters treating animal waste is especially attractive[4,5] since these streams can provide inexpensive (or free) nutrients.[6] In addition to macronutrients (C, N, and P), anaerobic digester effluents can also supply micro-nutrients and vitamins necessary for algal growth.[7,8] Integrated algal farms would thus mitigate nutrient release into the environment from animal operations.

N and P forms available in effluents from anaerobic digesters treating dairy waste are diverse and include both organic and inorganic compounds.[9,10] In addition, the effluents can contain organic carbon compounds such as volatile fatty acids (VFA) that have the potential to support heterotrophic growth of microalgae[2,11,12] and inorganic carbon (bicarbonate) for photosynthetic carbon fixation.[13] Typically, growth rates are higher when microalgae are able to utilize or assimilate the soluble carbon substrates available in the media since these are more bioavailable relative to soluble CO_2. Furthermore, metabolism of organic substrates can also generate additional reducing equivalents (and energy) for more rapid growth.[14] In summary, for high productivity in anaerobic digester effluents, microalgae must be efficient in utilizing available substrates (C, N, and P) without being negatively impacted by innate microorganisms.[15] However, anaerobically digested animal wastes are dark-colored and usually contain high concentrations of particulates that restrict the amount of light available to microalgae suspended within these growth media.[6,9,10,16] Dilution of the effluent could improve light penetration[5,17,18] and also solubilize some precipitated nutrients (such as PO_4^{-3}, Ca^{+2}, and Mg^{+2})[19] but, excess dilution could limit productivity due to nutrient limitations. Therefore, a balance between dilution (and thereby light penetration) and nutrient availability must be established to ensure high phototrophic productivity.[20]

In this study, native microalgae from a municipal wastewater treatment facultative lagoon were cultured and

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enriched in the effluent of an anaerobic digester treating dairy waste. Thereafter, the abundant alga was isolated and systematically cultivated in the anaerobically digested dairy waste (ADDW) to quantitatively assess growth and nutrient uptake at varying levels of incident irradiance. The results were used to correlate productivity of the isolate with utilization of inorganic and organic nutrient forms.

Materials and Methods

Growth medium, algal strain, and experimental setup

Anaerobic digester effluent samples were obtained from an Induced Blanket Reactor (IBR) treating dairy waste at the Wadeland Dairy, Ogden, UT. The anaerobic digester operates at slightly thermophilic conditions (40–45°C). Immediately after collection, the samples were diluted fourfold with DI water to (partially) solubilize mineral precipitates and centrifuged at 500*g* for 5 min to remove heavy and insoluble particulates and stored at −20°C. Our previous studies have shown that nearly 20% of the insoluble phosphates dissolve when the effluent is 4× diluted.[19] Prior to use, the frozen samples were thawed and further 5× diluted. For light penetration studies, the untreated (whole) effluent was centrifuged at 10,000*g* for 15 min and the supernatant was passed through a 0.45 μm filter to eliminate all particulates.

The algal strain used in the reported studies was an isolate from the Logan Wastewater Treatment Facility (LWTF) located in Logan, Utah. LWTF uses a facultative lagoon system to treat municipal wastewater through biological assimilation of nutrients, at least in part, due to natural proliferation of microalgae.[21] Microalgae-rich effluents from the LWTF were cultivated on ADDW to enrich for rapidly growing strains. Thereafter, sterile agar plates prepared with diluted and centrifuged (500*g* for 5 min) ADDW were inoculated with cultures from the enrichments using the spread-plate technique. A single microalgal colony that grew most rapidly on the solid media was further purified using a streak-plate technique. Successive transfers on plates were performed until unialgal colonies were confirmed through microscopic observation.

The algal isolate (named LLAI) was then grown in sterilized ADDW and used as inoculum for cultivation studies in non-sterile digester effluents. The concentration of algal biomass in inoculum used was 1.0–1.2 g L^{-1}; the ratio of inoculum to non-sterile effluent was 1:4. LLAI was cultivated (in non-sterile digester effluent) at various incident light intensities to systematically assess phototrophic nutrient utilization and biomass productivity. 1L spinner flasks (Corning, Tewksbury, MA; S/N-3561) containing digester effluents were placed on stir plates (Isotemp model 1110049S, Thermo Fisher Scientific, Waltham, MA) and stirred at 125 rpm at room temperature. The flasks were illuminated from two radially opposite directions using 40 W fluorescent lamps (Ecolux Sunshine 40, GE Lighting, East Cleveland, OH). In uninoculated flasks, light intensities were measured at the center of the flasks and at a height of 3.5″ from the bottom; liquid level in the flasks was approximately 7″ when containing 1L of media. All light measurements were performed using a spherical quantum microsensor (Model S-SQS/L, Heinz Walz GmbH, Effeltrich, Germany) and light meter (Model LI- 250A, LI-COR, Lincoln, NE).

Microalgal cultures were cultivated at five incident irradiance levels, that is, 255, 485, 745, 885, and 1,100 μmoles m^{-2} s^{-1} and a 12 h light–dark cycle was maintained throughout the experiment using a timer connected to the light circuit. At each irradiance level, growth experiments were performed in triplicate flasks. Initial pH of ADDW was measured to be 7.0–7.2. This pH was maintained during the cultivation experiments through CO_2 sparging. All the experiments were performed at room temperature (23–25°C). Controls included (i) uninoculated digester effluents to assess biological and/or chemical changes in the absence of algal strains and (ii) algae cultures incubated in dark to evaluate heterotrophic growth by algae or activity of native microbes, if any.

Analytical methods

Growth was monitored by periodically drawing culture samples and analyzing for total suspended solids (TSS) as outlined in Standard Method 2540D for examination of water and wastewater.[22] In addition, samples were filtered through 0.45 μm syringe filters and analyzed for dissolved ammonium nitrogen (NH_4^+-N)[23], total nitrogen (TN) (Standard Method 4500N-C), dissolved total phosphorous (TP) (Standard Method 4500P-I), ortho phosphorus (ortho-P) (Standard Method 4500P-E), and dissolved chemical oxygen demand (dCOD) (Standard Method 5220D).[22] Dissolved organic N (org-N) and organic P (org-P) were estimated by subtracting NH_4^+-N from TN and ortho-P from TP, respectively.[24]

Triacylglyceride (TAG) content of cells was determined using previously reported methods.[25] First, algal cells from experimental cultures were harvested by centrifugation and lyophilized. Thereafter, 200 mg of lyophilized cells were sonicated in a 1.5 mL solution of hexane, chloroform and tetrahydrofuran mixed in a ratio of 1:1:1 (v/v/v). Lipids extracted in the mixture were separated from cell debris by centrifugation. The extracts were analyzed using gas chromatography (model GC-15A, Shimadzu Scientific Instruments, Columbia, MD). Analytes were separated on a 15 m-long RTX-biodiesel column (Restek Corporation, Bellefonte, PA) and detected using a Flame Ionization Detector (FID). Helium was used as the carrier gas with a column flow rate of 2.73 mL min^{-1}. To quantify TAG concentration, FID detector response was first calibrated using appropriately diluted (0.1–2.5 mg mL^{-1} in hexane) triolein standards (Sigma-Aldrich, Allentown, PA). Triglyceride concentrations were estimated relative to triolein from calibration curves.

Optical microscopy

An optical microscope (Axioskop 40 equipped with Axiocam HR3 camera, Carl Zeiss Microscopy, LLC, Thornwood, NY) was used for morphological observation of LLAI cells. The objectives and contrast setting used for 400 and 1,000× magnifications were A-plan 40×/0.65 with phase-2 contrast and A-plan 100×/1.25 oil with phase-3 contrast, respectively.

Results and Discussion

Microscopy-based identification of the strain

Microscopic observations performed after repeated subculturing of the mixed culture from the Logan Wastewater Treatment Plant (Logan, UT) in ADDW suggested that the dominant cells in the enriched culture were spherical/ovoid shaped with a diameter between 1.5 and 3 μm. A closer look at algal cells under 1,000× magnification confirmed the

presence of thin cell wall and a cup or girdle shaped bright green single parietal chloroplast within the cells (see light microscopy image in Supplementary Information). When plated on solid media, the isolate (named LLAI) formed 0.05–0.2 cm grass green-colored colonies. These observations are consistent with previous morphological descriptions of *Chlorella* spp.[26,27] In addition, our previously reported 23S rRNA gene sequence of LLAI has suggested a 98% match with *Chlorella vulgaris*.[28] The results from the enrichment experiments suggest that through the process of continuous sub-culturing, the *Chlorella* sp. strain LLAI adapted best to the ADDW relative to other microalgae populations in the original inoculum from the wastewater treatment plant.[2,3,5,29] It is possible that LLAI grew more rapidly and was able to utilize nutrients better than other competing organisms.

Light penetration into ADDW

Before performing systematic cultivation studies, the attenuation of light (and thereby its availability for photosynthesis) within the ADDW was assessed. Penetration of light into anaerobic digester effluents can become restricted due to: (i) scattering of light by suspended solids and (ii) absorbance of light by colored organic compounds.[30,31]

Figure 1 shows the intensity of light detected in variously diluted unfiltered (whole) (Figure 1a) and filtered (Figure 1b) ADDW (vertical axes) relative to intensities measured in DI water (horizontal axes) at varying levels of incident irradiance. Thus, Figure 1a shows the effect on light penetration due to scattering and absorption of light by suspended solids as well as soluble (and colored) components present in the digester effluent at various dilutions and irradiance levels. Figure 1b shows light absorption due to soluble components alone. It can be seen from Figure 1a that there was no measurable penetration of light through whole effluents when dilutions were lower than 10×. Phototrophically significant light intensities (>100 μmoles m^{-2} s^{-1}) were detected only above 40× dilution of the whole effluent and when incident irradiance was >700 μmoles m^{-2} s^{-1}. The maximum recorded intensity of light within the unfiltered effluent was only 205 μmoles m^{-2} s^{-1} (at 40× dilution) when incident irradiance was 1,100 μmoles m^{-2} s^{-1} (Figure 1a). This means that during outdoor cultivation, phototrophic growth would be supported in the anaerobic digester effluents only if dilution of the medium exceeded 40× and solar irradiance exceeded 700 μmoles m^{-2} s^{-1}.[32,33] Understandably, this level of dilution would also entail higher water use.

Light penetration significantly improved when suspended solids were removed and a phototrophically favorable light intensity of >100 μmoles m^{-2} s^{-1} was measured at a much lower 10× dilution with an incident light of <700 μmoles m^{-2} s^{-1} (Figure 1b). However, >5× dilution was required, even in the absence of particulates, to allow significant penetration of light. In a large-scale application, it may be impractical to either use a high degree of dilution or remove all the suspended solids. Therefore, a combined approach of removal of coarse solids and partial dilution was used as a feed preparation strategy in the current study. A fourfold preliminary dilution of samples was first performed to (at least partially) dissolve mineral precipitates such as phosphates.[19] Thereafter, coarse solids were removed by settling. Finally, the decanted effluent was further diluted fivefold to decrease the concentrations of dissolved organic compounds and improve light penetration.

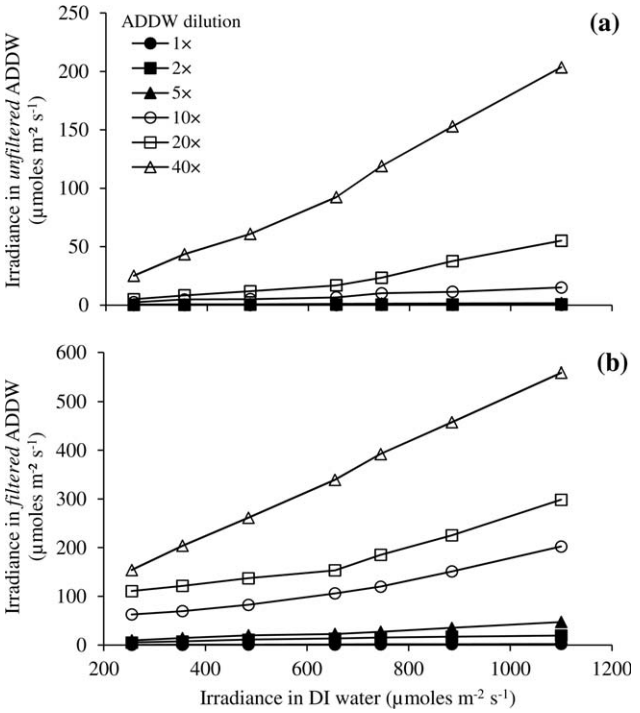

Figure 1. Penetration of light through the ADDW effluent at different levels of dilution: (a) whole effluent and (b) effluent with suspended solids removed.

Culture growth at varying levels of incident irradiance

Experimental controls that were incubated in the absence of LLAI (i.e., un-inoculated controls) did not show any measurable growth (increase in TSS) or change in elemental composition (data not shown). It is likely that the obligate anaerobes (such as methanogens) present in the digester were inactivated under aerobic conditions, while the facultative organisms (such as hydrolytic and acidogenic bacteria) were unable to further metabolize the organic carbon in the digestate.[34] Also, no algal growth was observed in controls inoculated with LLAI but incubated in the dark, indicating that strain LLAI was unable to grow heterotrophically in the absence of light.

To assess phototrophic growth in ADDW, LLAI cultures were grown at various light intensities and the measured biomass accumulations are shown in Figure 2. Maximum productivities were observed during days 6–12 and linearly increased with increasing light intensities (see inset to Figure 2). The maximum productivity during this period was assessed to be 0.34 g L^{-1} d^{-1} and occurred at the highest incident illumination level of 1,100 μmoles m^{-2} s^{-1}. Microalgae concentrations after 12 days of growth at this illumination were >3.2 g L^{-1}; final biomass concentrations (after 20 days of cultivation) exceeded 4.5 g L^{-1}. The culture concentrations and productivities reported in this study are significantly higher than reported by others that have cultivated *Chlorella* sp. on unsterile municipal or agricultural wastewaters.[2,3] Qin et al.[29] reported higher productivity (0.45 g L^{-1} d^{-1}) for *Chlorella* cultivated in bleach-sterilized anaerobic digester effluents, but yields were lower in unsterile or UV-sterilized waste (0.2–0.3 g L^{-1} d^{-1}). In any case,

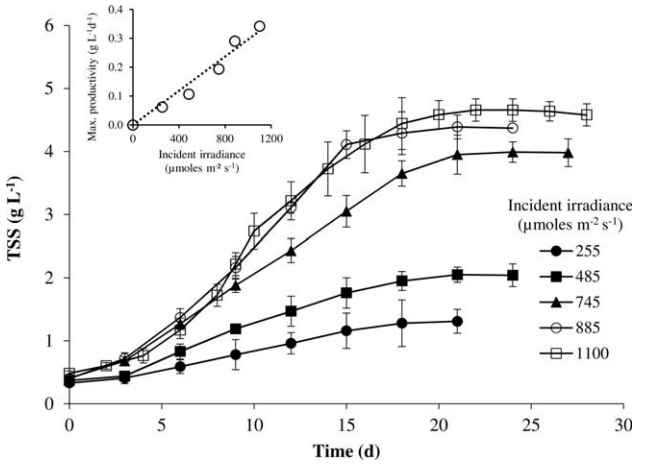

Figure 2. Growth of LLAI cultures under various levels of incident irradiance in the 20× diluted ADDW. Error bars indicate one standard deviation from mean values.

sterilization of anaerobic digester effluents on commercial scales to improve productivity may be cost prohibitive.

Results shown in Figure 2 also indicate that biomass productivities in highly illuminated cultures (i.e., at 11,00 μmoles m^{-2} s^{-1}) were more than fivefold higher than those cultivated at low levels of illumination (255 μmoles m^{-2} s^{-1}). Although incident illumination levels were high, the light intensities within the 20× diluted digestate (used as growth media) likely remained below 300 μmoles m^{-2} s^{-1} due to absorption/scattering of light within the media (Figure 1b). Thus, microalgae cells were able to grow without being photoinhibited. In other studies also, it was shown that *Chlorella* cultures were not photoinhibited when cultivated at high incident irradiance (1,500 μmoles m^{-2} s^{-1}) and a within-culture light intensity of nearly 300 μmoles m^{-2} s^{-1}.[35] LLAI cultures grown at lower light levels reached stationary phase much earlier than the cultures incubated at higher intensities, indicating insufficient availability of light to support prolonged phototrophic growth when irradiance is low.[36] At low incident light intensities, photons available for algal cells remain low as a significant fraction of incident photons are absorbed and/or reflected by particulates or colored compounds in the effluent medium.[18] The biomass productivity of 0.05–0.1 g L^{-1} d^{-1} at low intensities (below 500 μmoles m^{-2} s^{-1}) is consistent with productivities observed in other studies that used low illumination levels for cultivation.[5]

Nutrient uptake by LLAI culture

Table 1 shows the nutrient composition of the 20× diluted digested dairy waste used as the cultivation medium and Figure 3 shows nutrient consumption relative to LLAI biomass yields. These values were assessed from analyses of samples collected at the end of the growth cycle (i.e., final day samples collected between day 20 and day 30). It can be seen that TSS production at different levels of irradiance is linearly proportional to the removal of total-N (Figure 3a) and total-P (Figure 3b). From the slopes of the linear correlation between total-N removed and biomass (TSS) produced, the N content of biomass is estimated to be 5.7% (w/w). Similarly, the P content of biomass is calculated to be 0.7% (w/w). These values correspond closely to the expected N and P content of algae biomass with a general molecular formula of $C_{106}H_{263}O_{110}N_{16}P$ (molecular weight = 3,550 g mol^{-1}).[37]

Table 1. Composition of Dissolved Nutrients in 20× Diluted ADDW Used for Cultivation of *Chlorella* str LLAI

Component	Concentration (mg L^{-1})
Total nitrogen (TN)	349.8
Ammonium nitrogen (NH_4^+-N)	206.9
Organic nitrogen (org-N)	142.9
Total phosphorus (TP)	46.4
Orthophosphate phosphorus (ortho-P)	31.6
Organic phosphorus (org-P)	14.8
Soluble chemical oxygen demand (dCOD)	3,850

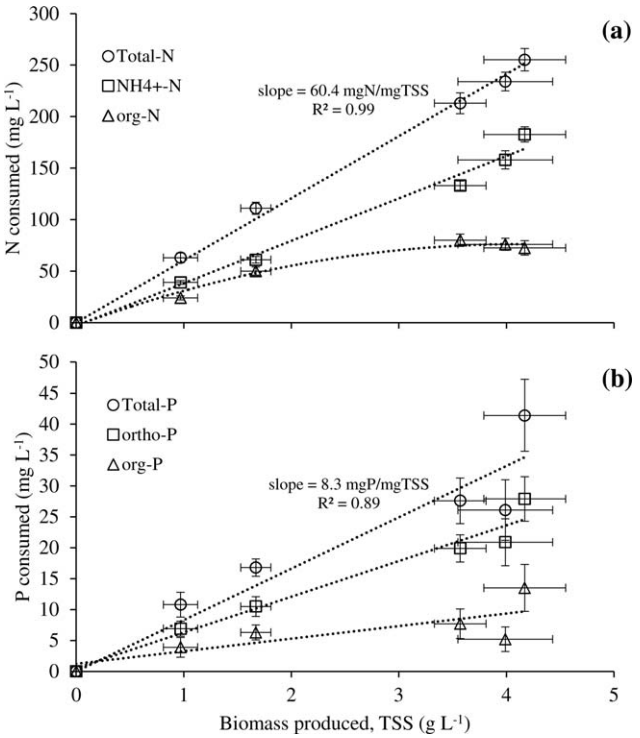

Figure 3. Comparison between nutrient uptake and final biomass concentration at various incident light intensities. (a) Shows N uptake relative to biomass produced and (b) shows P uptake relative to biomass produced. Error bars indicate one standard deviation from mean values.

Both organic and inorganic forms of N were consumed during growth. When biomass production was low (<2 g L^{-1}), both the organic and inorganic forms of N appear to be simultaneously utilized in nearly equal proportion—compare org-N and NH_4^+-N consumption at low biomass concentrations in Figure 3a. However, org-N consumption appears to plateau at values of approximately 70–80 mg-org-N L^{-1} likely due to uptake of all utilizable org-N present in the medium—equal to nearly half of the org-N present in the medium (Table 1). When higher amounts of biomass were produced (>2 g L^{-1}), it appears that growth was sustained by continued utilization of NH_4^+-N after depletion of utilizable org-N (see NH_4^+-N utilization at biomass concentration >2 g L^{-1} in Figure 4a). Under conditions that produced highest concentrations of biomass (incident illumination = 1,100 μmoles m^{-2} s^{-1}), nearly 90% of NH_4^+-N was removed.

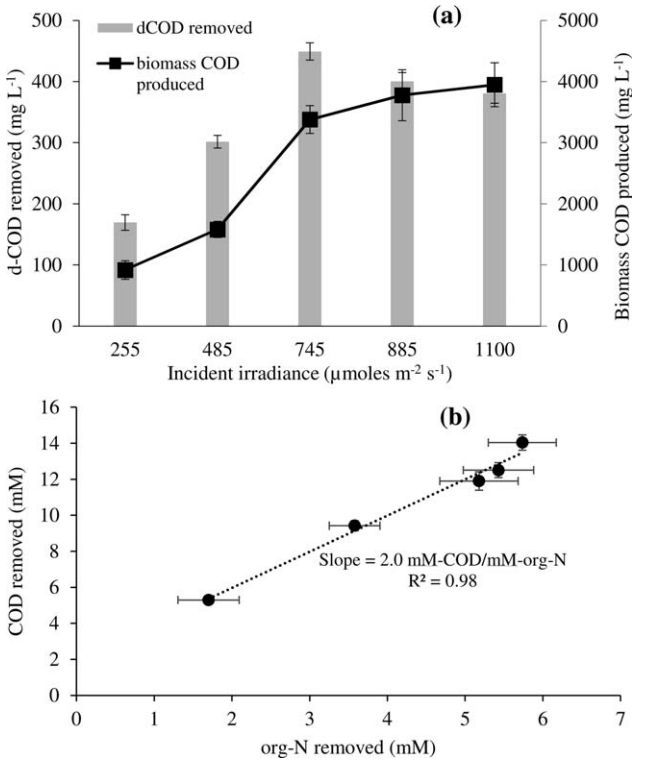

Figure 4. (a) Comparison of soluble COD removed and insoluble COD produced (biomass COD) due to LLAI growth in ADDW. (b) Correlation between decrease in soluble COD and uptake of org-N from solution due to growth of LLAI cultures. Error bars indicate one standard deviation from mean values.

Org-P and ortho-P were also simultaneously used (Figure 3b), although the significantly higher relative utilization of ortho-P suggests a preference for the inorganic P forms as a nutrient source. Nonetheless, nearly 90% of available org-P and ortho-P were ultimately utilized under conditions that resulted in highest growth.

The most likely forms of soluble org-N forms in anaerobically digested effluents are proteins, small peptides or amino acids, while sources of org-P could include nucleotides or other nucleoside phosphates (such as ATP). As such, uptake of org-N and org-P would also result in a simultaneous uptake of org-C. In phototrophic cultures, a small but measurable decrease in soluble COD was observed (see data in Figure 4a) and compared with initial COD values in Table 1), whereas no measurable dCOD removal was observed in control cultures incubated in the dark. It is thereby reasonable to infer that the dCOD decrease observed in phototrophic cultures likely occurred only in conjunction with org-N and org-P uptake. In addition, since org-N uptake was at least sevenfold higher than uptake of org-P (Figure 3), it can be deduced that a majority of dCOD decrease was due to org-N depletion from the media. Accordingly, as shown in Figure 4b, dCOD removal from solution was linearly proportional to depletion of org-N with a slope of 2 mol-dCOD mol N^{-1}.

Since the IBR digester (that processed the dairy waste) operates at 40–50°C, it is possible that soluble proteins were (at least partially) hydrolyzed within the reactor to smaller peptides and/or amino acids under the thermophilic conditions.[38] Table 2 shows the mol-dCOD·mol N^{-1} ratio for all the 20 amino acids calculated from the following equation that describes the balanced reaction for oxidation of an amino acid with a general chemical formula $C_nH_aO_bN_c$.

Table 2. Calculated COD Values and Molar COD/N Ratios of Amino Acids

Amino Acid	mol-COD mol N^{-1}	Averagemol COD/mol N
Glycine ($C_2H_5NO_2$)	1.50	2.64
Alanine ($C_3H_7NO_2$)	3.00	(for C2–C4 amino acids)
Serine ($C_3H_7NO_3$)	2.50	
Cysteine ($C_3H_7NO_2S$)	3.00	
Threonine ($C_4H_9NO_3$)	4.00	
Asparagine ($C_4H_8N_2O_3$)	1.50	
Aspartate ($C_4H_7NO_4$)	3.00	
Proline ($C_5H_9NO_2$)	5.50	5.09
Valine ($C_5H_{11}NO_2$)‡	6.00	(for >C5 amino acids)
Glutamine ($C_5H_{10}N_2O_3$)	2.25	
Glutamate ($C_5H_9NO_4$)	4.50	
Methionine ($C_5H_{11}NO_2S$)‡	6.00	
Isoleucine ($C_6H_{13}NO_2$)‡	7.50	
Leucine ($C_6H_{13}NO_2$)‡	7.50	
Lysine ($C_6H_{14}N_2O_2$)	3.50	
Histidine ($C_6H_9N_3O_2$)	1.67	
Arginine ($C_6H_{14}N_4O_2$)	1.38	
Tyrosine ($C_9H_{11}NO_3$)‡	9.50	
Tryptophan ($C_{11}H_{12}N_2O_2$)‡	5.75	

The ‡ symbols in first column indicate very hydrophobic amino acids.

$$C_nH_aO_bN_c + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3c}{4}\right)O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3c}{2}\right)H_2O + cNH_3. \quad (1)$$

The COD value of the amino acid is the stoichiometric coefficient of molecular oxygen (O_2) which is $(n+a/4-b/2-3c/4)$. Oxidation of N is ignored in Eq. 1 since dichromate, which was used as the oxidizing agent for the COD measurements, does not nitrify N. From calculated values shown in Table 2, only small amino acids (C1–C4) as well as glutamine, histidine and arginine have mol-COD·mole-N^{-1} ratios between 1.5 and 3. It can also be noted that the hydrophobic amino acids (marked with a ‡ symbol in Table 2)[39] are larger than the hydrophilic ones and may be less available for biological uptake. These hydrophobic amino acids have also large values of mol-COD mol N^{-1} (generally >6, Table 2). These calculations indicate that LLAI likely utilized N from C1–C4 amino acids or glutamine, histidine, and arginine but possibly did not uptake other large/hydrophobic amino acids and peptides. While we haven't found any literature that demonstrates the uptake of amino acids by microalgae, uptake of small peptides from soils by terrestrial plants has recently been demonstrated and is believed to play a significant role in the soil nitrogen cycle.[40]

The assimilation of the carbon associated with org-N and P is also expected to contribute to the generation of microalgae biomass (along with phototrophically fixed C). In addition to data showing the depletion of soluble COD (which is a measure of org-C uptake), Figure 4a also shows the calculated increases in biomass COD as a result of microalgae growth. The COD of algal cells were estimated from Eq.1 and by assuming a cell composition given by the molecular formula $C_{106}H_{263}O_{110}N_{16}P$. By these calculations, the COD of algal cells is approximately 0.946 g-COD per g-biomass. The results shown in Figure 4(a) suggest that at low illumination levels (255 and 485 μmoles m^{-2} s^{-1}), the depleted soluble COD is nearly 20% of the COD accumulated within biomass (compare values on the left and right ordinates in Figure 4(a)). At higher illumination levels (745, 885, and 1,100 μmoles m^{-2} s^{-1}), the dCOD removed is a smaller

fraction (nearly 10%) of the COD produced as algae biomass. These results are consistent with our discussion above (Figure 3a) that under low illumination conditions, org-N was a more significant portion of the total N utilized, which would result in higher relative uptake of org-C and incorporation into cellular material. When inorganic nitrate, ammonia or ortho-P are used as N and P sources, all cellular carbon must be phototrophically fixed, whereas cultures adapted to utilization of organic N and P forms (e.g., strain LLAI) can increase productivity by additional "heterotrophic" utilization of the C associated with the org-N and org-P. This trait is particularly useful in media such as animal waste where phototrophic growth is restricted by low transmission of incident light. In addition, microalgae cultures that are able to utilize diverse N and P forms would be suitable for more complete remediation of nutrient-rich waste streams. Studies with terrestrial plants suggest that heterotrophic C utilization, rather than N, is a more significant motive for uptake of peptides.[41] It is not clear if similar regulatory mechanisms are also present in microalgae; our results show that LLAI did not utilize the vast majority of the non-nitrogenous soluble COD. Rather, it appears that the primary organic C assimilation occurred only in conjunction with org-N and org-P uptake.

Organic P utilization has not been previously reported in phototrophic algal cultures,[2,3,5] although comparison of TN and NH_4^+-N uptake data in some reports would suggest that org-N assimilation likely did occur, but was not explicitly assessed.[29,42] In any case, LLAI appears to have effective mechanisms for capture of diverse N and P forms. Its original habitat, a municipal wastewater treatment lagoon, perhaps contained a similarly diverse nutrient pool. Overall, our results suggest that isolates from natural environments could be more successful in terms of N and P removal/remediation.

Triacylglycerides accumulation during cultivation in anaerobic digester effluent

To estimate the biodiesel potential of LLAI cells in the anaerobic digester effluent, intracellular triacylglycerides (TAGs) accumulation was monitored during the course of cultivation. Average values of maximum TAG contents measured under the various illumination conditions are shown in Figure 5. It was observed that accumulation of TAGs in algal cells started during exponential growth phase and plateaued at stationary growth phase (see inset to Figure 5). From these kinetic data for cultures illuminated at 1,100 μmoles m^{-2} s^{-1}, the lipid productivity over days 12–24 (after onset of lipid accumulation) was calculated to be 37 mg-TAG L^{-1} d^{-1}. Other studies on cultivation of *Chlorella* sp. in unsterile dairy waste have reported lower lipid productivities of 5–11 mg-TAG L^{-1} d^{-1}.[3,5,42] The improvement in lipid productivities over previous studies could be due to cultivation at higher light intensities.[43] However, cellular TAG content remained relatively low (<10% of cell mass), possibly since cultures were not nutrient deprived (see NH_4^+-N values in Table 1 and compare with NH_4^+-N utilization shown in Figure 3a) and lipid accumulation pathways were not fully triggered.[44] Higher dilutions might be able to facilitate more complete NH_4^+-N utilization and allow higher lipid productivities. Alternately, hydrothermal liquefaction could be used to convert the generated biomass to biocrude and ultimately to biofuels.

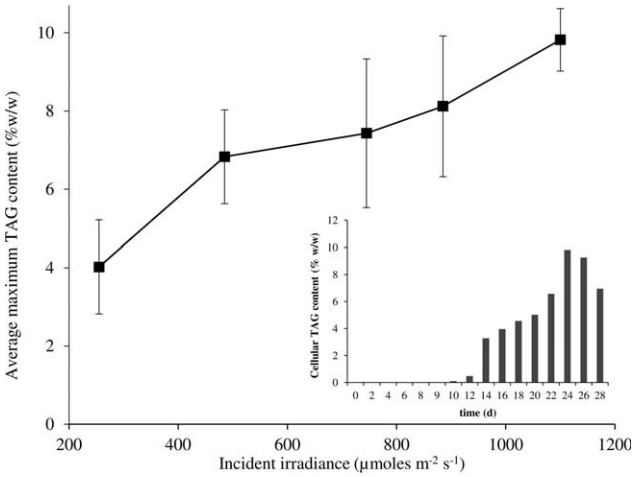

Figure 5. Maximum measured TAG content of LLAI cultures grown at various light intensities in 20× diluted ADDW. Inset shows transient changes in cellular TAG content for cultures cultivated at an incident irradiance of 1,100 μmoles m^{-2} s^{-1}. TAG accumulation starts at mid exponential phase (day 12) and reaches maximum values at stationary phase (day 24). Error bars indicate one standard deviation from mean values.

Conclusions

The *Chlorella* isolate (LLAI) from a municipal wastewater treatment lagoon grew photosynthetically in a 20× diluted ADDW and was shown to be versatile in utilizing both inorganic and organic forms of N and P. The supply of high incident irradiation (1,100 μmoles m^{-2} s^{-1}) significantly improved biomass and lipid productivities likely due to alleviation of light limitations within the cultures growing in a colored medium. Our results also suggest that while growth was phototrophic, the organic portions of the utilized nutrients were "heterotrophically" incorporated into the cellular material. Findings of this research study indicate that algal strains isolated from natural environments are likely well-suited for nutrient utilization/remediation and could also serve as productive feedstocks in systems that seek to integrate waste streams with biofuel production.

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