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Energy Demands of Nitrogen Supply in Mass Cultivation of Two Commercially Important Microalgal Species, *Chlorella vulgaris* and *Dunaliella tertiolecta*

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Abstract Mass culture of microalgae is a potential alternative to cultivation of terrestrial crops for bioenergy production. However, microalgae require nitrogen fertiliser in quantities much higher than plants, and this has important consequences for the energy balance of these systems. The effect of nitrogen fertiliser supplied to microalgal bubble-column photobioreactor cultures was investigated using different nitrogen sources (nitrate, urea, ammonium) and culture conditions (air, 12% CO_2). In 20 L cultivations, maximum biomass productivity for *Chlorella vulgaris* cultivated using nitrate and urea was 0.046 and 0.053 g L^{-1} day^{-1}, respectively. Maximum biomass productivity for *Dunaliella tertiolecta* cultivated using nitrate, urea and ammonium was 0.033, 0.038 and 0.038 g L^{-1} day^{-1}, respectively. In intensive bubble-column photobioreactors using 12% CO_2, maximum productivity reached 0.60 and 0.83 g L^{-1} day^{-1} for *C. vulgaris* and *D. tertiolecta*, respectively. Recycling of nitrogen within the photobioreactor system via algal exudation of nitrogenous compounds and bacterial activity was identified as a potentially important process. The energetic penalty incurred by supply of artificial nitrogen fertilisers, phosphorus, power and CO_2 to microalgal photobioreactors was investigated, although analysis of all energy burdens from biomass production to usable energy carriers was not conducted. After subtraction of the power, nitrogen and phosphorus energy burdens, maximum net energy ratios for *C. vulgaris* and *D. tertiolecta* cultivated in bubble columns were 1.82 and 2.10. Assuming CO_2 was also required from a manufactured source, the net energy ratio decreased to 0.09 and 0.11 for *C. vulgaris* and *D. tertiolecta*, so that biomass production in this scenario was unsustainable. Although supply of nitrogen is unlikely to be the most energetically costly factor in sparged photobioreactor designs, it is still a very significant penalty. There is a need to optimise both cultivation strategies and recycling of nitrogen in order to improve performance. Data are supported by measurements including biochemical properties (lipid, protein, heating value) and bacterial number by epifluorescence microscopy.

Keywords Algae · Photobioreactor · Nitrogen fertiliser · Energy balance · Biofuel

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Introduction

In order to reduce the environmental impacts of fossil fuel usage, to provide energy security and to meet international- and national-set government targets, there is an urgent need to develop sustainable energy technologies [3, 21]. Bioenergy derived from crops has the potential to fulfil at least some of this requirement and already has commercial applications in the transport fuel and power industries [33, 63].

One potential method of producing biomass for energy and greenhouse gas mitigation that is not currently in large-scale commercial production is the cultivation of microalgae

in place of conventional terrestrial crops [12, 18]. Microalgal mass cultures have several process advantages over their terrestrial counterparts including high areal productivity and photosynthetic efficiency, high cellular concentrations of lipids that can be converted to bio-oil, and the potential to offset power plant CO_2 emissions [18, 29, 38, 64, 72, 73]. It has been calculated that the requirement for large areas of land for growing terrestrial energy crops could make microalgae a more appropriate solution for meeting biomass production requirements [12].

All commercial and potential biofuel technologies (including, for example, corn ethanol and soy biodiesel) face challenges in terms of their life-cycle 'net benefits', when the energetic and environmental costs incurred during production are accounted for [30]. Despite this, fuels such as corn ethanol and soybean diesel do seem to offer a positive energy return at present, with net energy balances of 1.25 and 1.93, respectively [30]. The potential use of microalgal technologies to provide bioenergy is often justified by the environmental benefits, particularly reduction in greenhouse gases released to the atmosphere [2, 56]. However, several life-cycle analyses have shown that, at present, technologies used to cultivate microalgae are not sustainable [4, 14, 45]. This is particularly the case for closed photobioreactors, which have much higher cultivation energy inputs than raceway ponds [39, 69].

In a recent analysis of algal bioenergy production, it was shown that the energy return on investment was below unity for each of the modelled scenarios, making current algal technologies uncompetitive with commercial fuels [4]. The same authors identified that cultivation accounted for 96.2% of the total energy investment for algal biocrude production, with harvesting, cell lysing and lipid separation accounting for just 0.9%, 0.2% and 2.7% of the total energy investment. As a result, the most significant improvements in the performance of algal bioenergy technologies are likely to come from managing and improving the energetic and environmental burdens associated with biomass production [4, 14, 45].

A fundamental issue that has been largely overlooked in the most recent drive towards commercial microalgae production is the need to supply large amounts of essential nutrients to grow microalgae in any cost-effective, commercially viable way [12, 69]: If microalgae are to be used for large-scale transport fuel production, it is vital to consider the demands on fertiliser supply, especially the nitrogen and phosphorus sources [56]. In baseline modelling studies, it is logically assumed that nutrients/fertilisers will be manufactured [12, 14] because this is the mechanism that currently supports the majority of global agriculture [61]. However, more recent studies have demonstrated the need to conserve and recycle essential nutrients and carbon, in order to improve the energetic efficiency and fulfil sustainability criteria [4, 16].

Supply of nitrogen to microalgal cultivations is significant because the manufacture of nitrogen fertiliser via ammonia (Haber–Bosch process) is energy-intensive [15]. Further, microalgal biomass typically has a high protein content (up to 60%) with an optimal molar carbon/nitrogen ratio of approximately 6.6:1 [24, 48]. This compares to terrestrial biomass feedstocks which have lower nitrogen demands, their C/N ratios typically ranging between 20:1 and 120:1 [23]. The fertiliser demands for algae are thus potentially higher than for other crops. Production of nitrogen fertilisers requires more energy per unit mass than phosphorus fertilisers [4, 15], and microalgae require around seven times higher mass of nitrogen than phosphorus [24]. However, although nitrogen supply may have the greatest energy burden, the need to supply large quantities of phosphorus from mineral rock is likely to be unsustainable, and phosphorus may be a more limited resource in this respect [56]. Life-cycle assessments of microalgal technologies have invariably identified the importance of minimising the consumption of manufactured fertilisers: For example, Clarens et al. [14] identified the need to supply wastewater to provide nutrients, whilst Stephenson et al. [69] calculated that recycling the nutrients via anaerobic digestion greatly improved process efficiency. Similarly, Beal et al. [4] and Pate et al. [56] each highlight the need to recycle nutrients in order to improve energetic efficiency and competitiveness with conventional fuels.

Some cyanobacteria are capable of fixing their own nitrogen from N_2, but most commercially valuable microalgae must be provided with external sources of nitrogen compounds. Typically, nitrogen is supplied in the form of nitrate (NO_3^-) and/or ammonium (NH_4^+) [13, 68, 71]. However, some species are capable of utilising urea [32], which may be useful for commercial applications due to its lower cost than nitrate compounds [68]. Assimilation of amino acids is possible [58], but heterotrophic cultivation strategies rely upon another source of organic matter to support them and are not considered in this work. The nitrogen source and dissolved CO_2 concentration can affect the biochemical composition, including lipid synthesis, and nitrogen limitation is a strategy for producing lipid-rich biomass [37, 54, 75].

The fate of nitrogen compounds in microalgal mass cultures has received relatively little consideration. The release of dissolved substances from microalgal cells, including nitrogenous compounds, is widely recognised [9]. In particular, the role of bacteria in intensive algal cultivation systems is still poorly understood, although recent research has characterised the bacterial community dynamics associated with microalgal cultivation [43].

The purpose of the research was to (a) measure the productivity of *Chlorella vulgaris* and *Dunaliella tertiolecta* cultivated using different nitrogen sources (nitrate, ammonium and urea), including the effects on calorific value and

biochemical composition (lipid, protein) content; (b) to measure the nitrogen dynamics and growth of microalgae in intensive bubble-column photobioreactors using flue gas concentrations of CO_2 and (c) to examine the effect of nitrogen energy inputs on the net energy return of bubble-column cultivations. Note that the investigation of energy consumption in this work examines only the biomass production component; downstream processing of biomass is not considered here.

Methods

Cultivation Conditions

Two experimental photobioreactor systems were used in this study: large 20-L polythene bioreactors and smaller-scale 1.4-L bubble-column reactors. It is important to note that in the descriptions that follow the nutrient, light and growth conditions were quite different between the two systems. In both bioreactor systems, water losses by evaporation were corrected daily by addition of ultra-pure water.

Polythene Photobioreactors (20 L)

Polythene photobioreactors containing 20 L working volume of microalgal culture, diameter 160 mm, were sparged with air (0.04% CO_2 approximately) at 10 L min^{-1} [34]. Light was provided at an incident irradiance of 225 μmol photons m^{-2} s^{-1} of photosynthetically active radiation (Li-Cor 190SA sensor) using eight cool white fluorescent tubes using continuous light. *C. vulgaris* and *D. tertiolecta* were each batch-cultivated for 14 days using nitrate, urea and ammonium as the nitrogen sources. Details of each of the treatments are shown in Table 1. The inoculum consisted of exponentially growing cells supplied with nitrate. Typically no acclimation period is needed for the nitrogen species tested [20]. The authors observed that exponential growth after transfer to different nitrogen sources was comparable. The temperature was 24 ±2°C during cultivation. Nutrient media for *D. tertiolecta* were according to 2ASW (Culture Collection of Algae and Protozoa, Oban, UK) formulation (milligrams per litre): $NaNO_3$ (112.5), Na_2HPO_4 (4.5), K_2HPO_4 (3.8), and artificial seawater (Ultramarine Synthetica, 30 g L^{-1}). Nutrient media for *C. vulgaris* were according to M8 formulation (milligrams per litre): KNO_3 (134), KH_2PO_4 (33), $Na_2HPO_4.2H_2O$ (12), $CaCl_2.2H_2O$ (0.6), $FeSO_4.7H_2O$ (5.8), $MgSO_4.7H_2O$ (18), $MnCl_2$ (0.2), $CuSO_4$ (0.1), and $ZnSO_4$ (0.1). The nitrate in each of these media was substituted for ammonium and urea such that a constant 1.33 mmol L^{-1} nitrogen was available in each of the treatments. The N/P ratios for standard *D. tertiolecta* and *C. vulgaris* media were used (Table 1). Media were made up using 0.2-μm filtered water (Millipore).

Intensive Bubble Columns (1.4 L)

These reactors had an internal diameter of 32 mm and received light from cool white fluorescent tubes at an

Table 1 Details of experiment treatments and tests conducted

Test	Species	Nitrogen Source	N/P ratio (mol)	CO_2 added	Analyses	Cultivation parameters
1	*Dunaliella tertiolecta*	Nitrate	24.9	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
2	*Dunaliella tertiolecta*	Urea	24.9	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
3	*Dunaliella tertiolecta*	Ammonium	24.9	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
4	*Chlorella vulgaris*	Nitrate	4.5	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
5	*Chlorella vulgaris*	Urea	4.5	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
6	*Chlorella vulgaris*	Ammonium	4.5	Air	DW, lipid, CHN, DOC, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, bacterial count	pH, DO, temperature
7	*Dunaliella tertiolecta*	Nitrate	16	12% (20.7 g day^{-1})	DW, CHN, HHV, DOC, DON, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}	pH, DO, temperature
8	*Dunaliella tertiolecta*	Nitrate	16	0.04% (0.07 g day^{-1})	DW, CHN, HHV, DOC, DON, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}	pH, DO, temperature
9	*Chlorella vulgaris*	Nitrate	4.5	12% (20.7 g day^{-1})	DW, CHN, HHV, DOC, DON, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}	pH, DO, temperature
10	*Chlorella vulgaris*	Nitrate	4.5	0.04% (0.07 g day^{-1})	DW, CHN, HHV, DOC, DON, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}	pH, DO, temperature

DW dry weight, *CHN* elemental composition, *DOC* dissolved organic carbon

incident irradiance of 350 μmol photons m^{-2} s^{-1} photosynthetically active radiation. The short light path was selected for high productivity, in order to maximise bioenergy production [35]. Four treatments are presented in this work: *C. vulgaris* and *D. tertiolecta* each cultivated using air (0.04% CO_2) and coal-fired plant flue gas concentrations of CO_2 (12% CO_2, 20% O_2, balance N_2), each in triplicate. Gas was sparged at a rate of 0.24 L min^{-1}, supplied pre-mixed from a cylinder (BOC special products, UK). Real flue gases containing concentrations of CO_2 in this range have been used successfully to cultivate *C. vulgaris* and *D. tertiolecta* in previous studies [19, 72].

The nutrient media used for *D. tertiolecta* were according to Carballo-Cardenas et al. [8], but with the bicarbonate removed to prevent a potential confound in inorganic carbon availability (milligrams per litre): KNO_3 (1010), $NaH_2PO_4 \cdot H_2O$ (138), $Na_2EDTA \cdot 2H_2O$ (14.4), $FeCl_3 \cdot 6H_2O$ (3.95), $CuSO_4 \cdot 7H_2O$ (0.04), $ZnSO_4 \cdot 7H_2O$ (0.05), $CoCl_2 \cdot 6H_2O$ (0.01), $MnCl_2 \cdot 4H_2O$ (0.24) and Na_2MoO_4 (0.01). Media for *D. tertiolecta* were adjusted to a salinity of 30 using artificial seawater formulation (Culture Collection of Algae and Protozoa, Oban, UK). *C. vulgaris* was cultured using M8 formulation according to Mandalam and Palsson [51] (milligrams per litre): KNO_3 (1010), KH_2PO_4 (249), $Na_2HPO_4 \cdot 2H_2O$ (88), $CaCl_2 \cdot 2H_2O$ (4.4), Fe EDTA (3.4), $FeSO_4 \cdot 7H_2O$ (44), $MgSO_4 \cdot 7H_2O$ (135), $Al_2(SO4)_3 \cdot 18H_2O$ (1.2), $MnCl_2 \cdot 4H_2O$ (4.4), $CuSO_4 \cdot 5H_2O$ (0.6) and $ZnSO_4 \cdot 7H_2O$ (1.1). For comparability, the nutrient media were each standardised proportionally to a common 10 mmol L^{-1} concentration of inorganic nitrogen. Temperature was controlled at 26±0.5°C using air conditioning.

Analytical Methods

Biomass Properties

Microalgal dry weight was analysed by vacuum filtration using Whatman GF/F filters (0.7 μm nominal pore size) and drying at 80°C for 24 h. The initial and final filter weights were measured using a six-point balance and dry mass calculated as $d=(w_i-w_f)/v$, where d is the dry weight (grams per litre), w_i and w_f are the dry filter weights before and after filtration, respectively, and v is the culture volume filtered (litres). Samples of *D. tertiolecta* retained on filters were washed three times using ammonium formate (0.5 M) to remove extracellular salt. Cell counts were conducted using a haemocytometer.

The elemental composition (carbon, hydrogen, nitrogen) of the biomass was measured using an elemental analyser (Thermo-Electron EA1112). Samples were extracted by centrifugation (1,000 rcf, 10 min) and dried at the same temperature as the dry weight measurements (80°C) for 48 h. The protein composition of the algal biomass was calculated by multiplying the total elemental nitrogen content by 4.44 (Lopez et al. 2010). The elemental composition (carbon, hydrogen, nitrogen) was used to calculate the higher heating value of the biomass by proximate analysis. This was derived from a multiple linear regression model of elemental composition vs. higher heating value for the same species cultivated in the same conditions [35]. The equation used was: HHV (kJ g^{-1})=−4.90+0.069 N+0.533 C+ 0.226H, where C, H and N are the percentage of carbon, hydrogen and nitrogen in the biomass.

The total lipid content was measured using modified Bligh and Dyer [5] solvent method. A biomass pellet for a single lipid sample was collected by centrifugation of 250 mL of culture (1,000 rcf, 10 min). Cells were disrupted in pre-weighed glass centrifuge tubes using sonication (Branson Digital Sonifier) at 13.5 W/10 mL of sample. *D. tertiolecta* required 1 min to ensure complete disruption (checked using microscope), but *C. vulgaris* required 30 min and cooling with an ice bath to ensure an equivalent result. Biomass was then dried (80°C, 72 h), weighed at room temperature and the lipid extracted using chloroform/methanol/water in the proportion 1:2:0.8. Adjustment of the ratio to 2:2:1.8 ensured phase separation, before the chloroform/lipid fraction was transferred to a micro-centrifuge tube and centrifuged at 11,000 rcf for 5 min to remove remaining cell debris. The chloroform was then evaporated using a heating block. Lipid weight was expressed as a percentage of the dry weight.

Nitrogen and Phosphorus Measurements

For cultivations using 20-L photobioreactors with alternative nitrogen sources, dissolved nitrate, ammonium, urea and nitrite were measured every 48 h. For cultivations using intensive bubble columns nitrate, ammonium and nitrite were also measured in these cultivations every 48 h. Dissolved nutrients were measured after filtration through Whatman GF/F filters. Analysis of NO_3^-, NO_2^- and PO_4^{3-} was conducted using standard colorimetric methods [27] using a Lachat Instruments Quick-Chem flow-injection autoanalyser. Ammonium (NH_4^+) was analysed using the method of Holmes et al. [31] using a Hitachi F2000 fluorescence spectrophotometer. Urea was measured using the diacetyl monoxime method of Mulvenna and Savidge [52], adapted for 5 mL sample sizes.

Dissolved Organic Matter

Dissolved organic matter was measured using samples filtered through Whatman GF/F filters (0.7 μm nominal pore size). In 20-L photobioreactors, dissolved organic carbon (DOC) was measured using a TOC-5000 Analyzer (Shimadzu) according to the Finnish standard SFS-ISO 8245.

Dissolved organic carbon samples from bubble-column photobioreactors were measured using a MQ1001 TOC analyser according to Qian and Mopper [60]. Dissolved organic nitrogen (DON) was measured in bubble-column photobioreactors at days 8 and 10 in flue gas treatments only. Analysis at other times was not possible due to the presence of high concentrations of nitrate that reduced analytical accuracy. DON was measured by subtraction of dissolved inorganic nitrogen from the total dissolved nitrogen, the latter measured by peroxodisulphate oxidation and UV radiation at pH 9 and 100°C [41].

F_v/F_m, Temperature, Dissolved Oxygen, pH and Bacteria

The performance of photosystem II was measured by the maximum quantum yield (F_v/F_m) with a pulse amplitude modulation (PAM) fluorometer (Water PAM, Walz, Germany). Temperature, dissolved oxygen and pH were each measured using a HACH HQ40d portable multimeter, temperature and pH with a HACH PHC101-03 IntelliCAL™ probe and DO with a HACH LDO101-03 IntelliCAL™ probe. Bacterial abundance was determined by staining with DAPI and examination using epifluorescence microscopy [34].

Statistical Analysis

The dry weight productivity was calculated using the equation $y=(d_i-d_f)/t$, where y is the growth rate (grams per litre per day), d_i and d_f are the concentrations (grams per litre) at two time points and t is the time between measurements (days). Nitrogen uptake rates were calculated from the dissolved measurements in the same manner using the dissolved inorganic nitrogen concentration (including urea). Treatments were compared where appropriate with two-sample and paired t tests using Minitab v.14. An effect was considered significant at the 5% level. Details of each of the cultivation tests conducted and respective analyses are presented in Table 1.

Results and Discussion

Nitrogen Sources

Growth and Nitrogen Uptake of D. tertiolecta

D. tertiolecta cultivations supplied with ammonium, nitrate and urea reached 0.48 to 0.54 g L^{-1} dry weight at day 14 (Table 2). These values are comparable with the published literature considering the light path length, air concentration of CO_2 (0.04%) and irradiance [11]. The productivity and cell density were comparable to similarly operated photobioreactors [13] and to high-rate algal ponds [65]. Data presented in Fig. 1a indicate that dry weight at day 14 was the lowest in the ammonium treatment. Maximum uptake of nitrogen from the medium ranged from 2.5 (SD ±0.1) to 3.8 (SD ±0.2) mg N L^{-1} day^{-1} for *D. tertiolecta*, shown in Table 2. Uptake of nitrogen in the urea treatment was significantly greater than the ammonium treatment (t=9.36, p=0.003), although there was no significant difference in maximum nitrogen uptake between ammonium and nitrate (t=2.29, p=0.15) or between nitrate and urea (t=3.13, p=0.052).

Growth and Nitrogen Uptake of C. vulgaris

Growth of *C. vulgaris* supplied with ammonium was healthy for the first 2 days but invariably collapsed between 3 and 4 days from the start of cultivation despite three repeated attempts (n=9 photobioreactors). Their demise was associated with a drop in pH, likely caused by nitrification and/or uptake of NH_4^+ (see "*C. vulgaris* Accessory Measurements"). The maximum dry weight attained by *C. vulgaris* treatments was approximately the same in nitrate and urea treatments (Table 2; Fig 1b). The mean maximum growth rate (days 2–6) using nitrate was 12% lower than when urea was used, although there was no significant difference between treatments (t=0.84, p=0.489). Table 2 shows that the maximum uptake of dissolved nitrogen (days 2–6) in nitrate and urea treatments was comparable,

Table 2 Effect of treatment (species and nitrogen source) on maximum dry weight growth rate, maximum nitrogen uptake rate (each obtained between days 2 and 6) and maximum dry weight density (at day 14)

Species	N Source	Max dry weight growth rate (days 2–6) (g L^{-1} day^{-1})	Max dry weight (day 14) (g L^{-1})	Max N uptake rate (days 2–6) (mg L^{-1} day^{-1})
Dunaliella tertiolecta	Nitrate	0.033 (0.010)	0.52 (0.07)	3.1 (0.4)
Dunaliella tertiolecta	Urea	0.038 (0.007)	0.52 (0.06)	3.8 (0.2)
Dunaliella tertiolecta	Ammonium	0.038 (0.004)	0.48 (0.02)	2.5 (0.1)
Chlorella vulgaris	Nitrate	0.046 (0.003)	0.53 (0.03)	3.2 (0.2)
Chlorella vulgaris	Urea	0.052 (0.012)	0.54 (0.06)	3.5 (0.4)

Data are mean (± standard deviation)

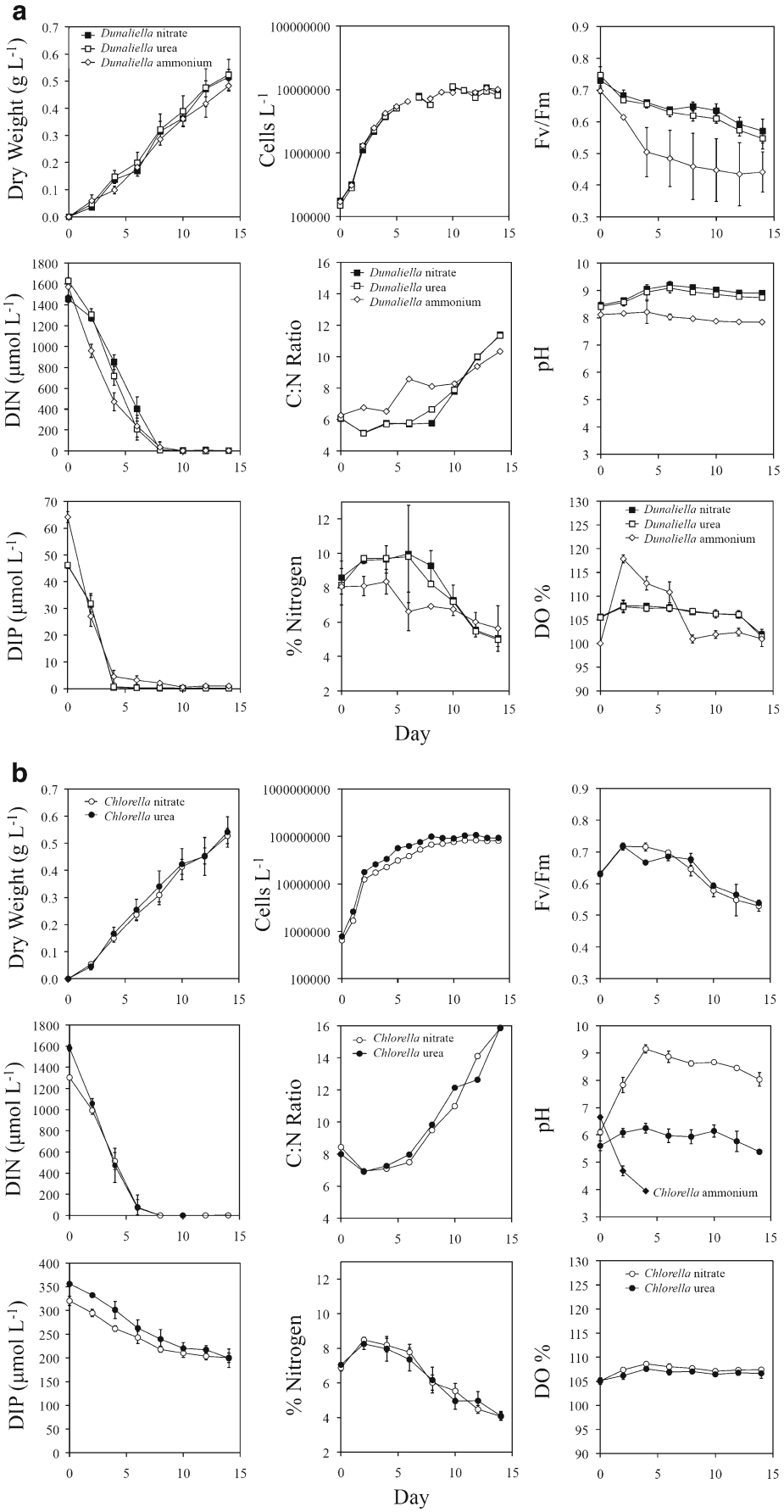

Fig. 1 **a** Parameters measured during cultivation of *D. tertiolecta* in 20-L photobioreactors using either nitrate, urea or ammonium as the nitrogen source. For each point, $n=3$, and *error bars* are standard deviations. **b** Parameters measured during cultivation of *C. vulgaris* in 20-L photobioreactors using either nitrate or urea as the nitrogen source. For each point, $n=3$, and *error bars* are standard deviations

and there was no significant difference between treatments (t=0.9, p=0.462). When all treatments of both species were combined, there was positive correlation between maximum growth rates and maximum uptake of supplied nitrogen (r^2=0.322).

To summarise, cultivation of either species using urea offered a small but non-significant advantage over nitrate in terms of maximum growth rate and/or maximum biomass attained. In the context of industrial biomass production, there thus seems little to choose between the use of urea, nitrate and in the case of *D. tertiolecta*, ammonium. However, it should be noted that nitrogen source may impact biochemical composition, especially the lipid classes present [49]. Ammonium is unlikely to be an appropriate nitrogen source for *C. vulgaris* (and likely many other freshwater species) where high cell densities and un-buffered solutions are used.

D. tertiolecta Biomass Composition

All treatments for both species remained nitrogen-replete between days 2 to 6, and this is reflected in the nitrogen content and C/N ratios presented in Fig. 1a. The nitrogen content of *D. tertiolecta* during nutrient-replete growth (day 4) ranged from 8.35% (SD ±0.72%) to 9.77% (SD ±2.13%). Sources of nitrogen in the media were exhausted between days 6 and 8 in all treatments. By the end of the cultivation period, the nitrogen content of *D. tertiolecta* biomass was depleted to approximately half of the optimal values (Fig. 1a).

Biochemical properties for specific parts of the cultivations are shown in Table 3. Day 4 corresponds to nutrient-replete conditions, day 8 corresponds to the point of nitrogen exhaustion and day 14 is the nitrogen-deprived stage at the end of the experiment. The protein content decreased from 37% to 43% at day 4 to 22—25% by day 14. The calorific values of *D. tertiolecta* showed a minimal increase over the same period, and there was little evidence for any lipid accumulation toward the end of the experiment (Table 3). Thus, by deduction, the primary response to nitrogen limitation by *D. tertiolecta* was carbohydrate assimilation. Chen et al. [11] identified values of 15% to 18% lipid per unit dry mass for nutrient-replete and deprived *D. tertiolecta* biomass, respectively, and comparable values were obtained in this work (15% to 19%).

Chen et al. [11] reported that nitrogen limitation resulted in rapid accumulation of neutral lipids, whereas Lombardi and Wangersky [47], Gordillo et al. [26] and Sheehan et al. [65] detected no N limitation induced increase in lipid content of *D. tertiolecta*, *Dunaliella viridis* and *Dunaliella* sp. Gordillo et al. [26] showed that proportion of TAGs increased under N limitation if cultures were bubbled with air enriched with 1% CO_2, but no clear difference was seen under atmospheric CO_2 levels. Chen et al. [11] conducted their cultivations at 4% CO_2, whereas Lombardi and Wangersky [47] and polyethene bioreactor cultivations of this study were conducted at atmospheric CO_2 levels. Thus, the different CO_2 concentrations in the supplied gas may have been the cause for different responses to nitrogen limitation.

C. vulgaris Biomass Composition

The mean nitrogen content of *C. vulgaris* at day 4 was 8.18% (0.40%) and 7.97% (0.71)% for nitrate and urea treatments, respectively (Fig. 1b). Protein concentrations (Table 3) followed a similar dynamic to the *D. tertiolecta* cultivations. However, both the calorific value and lipid content increased in response to nitrogen limitation in the *C. vulgaris* treatments (Table 3). Paired t tests between days 4 and 14 identified a significant increase in lipid content (t≥2.8, p≤0.047) and heating value (t≥3.7, p≤0.004). Samples for both species contained less than 40% lipid, and evidence from Sialve et al. [66] suggests that it

Table 3 Biochemical properties of *Dunaliella tertiolecta* and *Chlorella vulgaris* (lipid, protein and heating value) at days 4, 8 and 14 of the cultivations

Species	N source	Day 4			Day 8			Day 14		
		Protein (%)	Lipid (%)	HHV (kJ g^{-1})	Protein (%)	Lipid (%)	HHV (kJ g^{-1})	Protein (%)	Lipid (%)	HHV (kJ g^{-1})
D. tertiolecta	Nitrate	42.5 (3.8)	16.0 (8.7)	22.5 (0.4)	41.2 (4.7)	14.9 (1.8)	21.5 (0.8)	22.4 (2.1)	14.6 (1.2)	23.2 (0.3)
D. tertiolecta	Urea	43.1 (1.3)	18.2 (0.8)	22.0 (0.1)	36.5 (6.5)	18.2 (0.8)	21.1 (0.6)	22.1 (2.3)	17.9 (3.4)	22.1 (0.4)
D. tertiolecta	Ammonium	37.1 (3.2)	18.6 (8.8)	22.7 (0.9)	30.7 (0.4)	18.6 (8.8)	22.8 (0.3)	25.0 (5.9)	14.7 (0.7)	22.9 (0.5)
C. vulgaris	Nitrate	36.3 (1.8)	21.0 (1.6)	23.8 (1.2)	26.7 (1.9)	18.4 (1.6)	23.1 (1.3)	18.1 (1.0)	27.0 (1.5)	26.6 (0.5)
C. vulgaris	Urea	35.4 (3.2)	20.9 (2.3)	23.6 (0.3)	27.3 (3.3)	22.3 (5.7)	24.5 (1.2)	18.1 (1.1)	27.9 (3.6)	26.8 (0.9)

Day 4 corresponds to nutrient-replete growth, day 8 the approximate point of nitrogen exhaustion and day 14 the experiment end point. Data are mean (± standard deviation)

would be energetically favourable to subject the whole biomass to anaerobic digestion, rather than to first extract the lipid for biodiesel production. However, Sialve et al. [66] only consider lipid extraction using dried biomass, which requires a considerable energy investment. Alternative dry and wet extraction methods may prove to be more efficient, especially if waste heat can be used to improve process efficiency [76]. Although only total lipids were measured in this work, it is important to note that the lipid profile/composition is also important for biodiesel production [38].

Apart from species-specific differences, the different responses of the two species to nitrogen limitation may be attributable to the nutrient media compositions used. Differences in nutrient stoichiometry may cause significant physiological effects on cell biochemistry, including lipid, protein and carbohydrate content, plus the classes of those compounds [24]. *D. tertiolecta* utilised all of the available inorganic phosphorus within 4 days of cultivation, but *C. vulgaris* remained phosphorus-replete throughout (Fig. 1). Part of this discrepancy in phosphate utilisation may be the accumulation of polyphosphates by *D. tertiolecta*, so that phosphate depletion in the media might have a negligible effect on growth [11, 59].

Dissolved Organic Carbon and Bacterial Cell Number

With the exception of the urea treatments, DOC is the pool of organic carbon exuded from microalgal cells. It is a loss of harvestable organic matter and a substrate for bacterial growth. Production of DOC is also driven by nutrient stress, especially supply of nitrogen [34]. The concentration of DOC in 20-L photobioreactors using alternative nitrogen sources is shown in Fig. 2. These figures are absolute values, but it should be noted that the urea treatments contained 0.665 mmol L^{-1} of added organic carbon at the start. Taking this into account, the difference in DOC concentrations between treatments was minimal, although production of DOC in the ammonium treatment between days 2 to 6 was higher than in the corresponding nitrate and urea treatments. The highest DOC concentration in *D. tertiolecta* treatments was attained in the ammonium treatments with mean 1.69 (SD ±0.47) mmol L^{-1}, which was 9% of the total organic carbon in the system. For *C. vulgaris*, the highest DOC concentrations were observed at the end of cultivation using urea (Fig. 2). In this case, the mean DOC accounted for 7.4% of the organic carbon in the photobioreactor.

Cultivations resulted in similar bacterial dynamics in nitrate and urea treatments for each species, with bacterial cell number increasing over the duration of the experiment (Fig. 3). In contrast, bacterial cell numbers in the *D. tertiolecta* ammonium treatment were much higher than the corresponding nitrate and urea treatments. By the end of

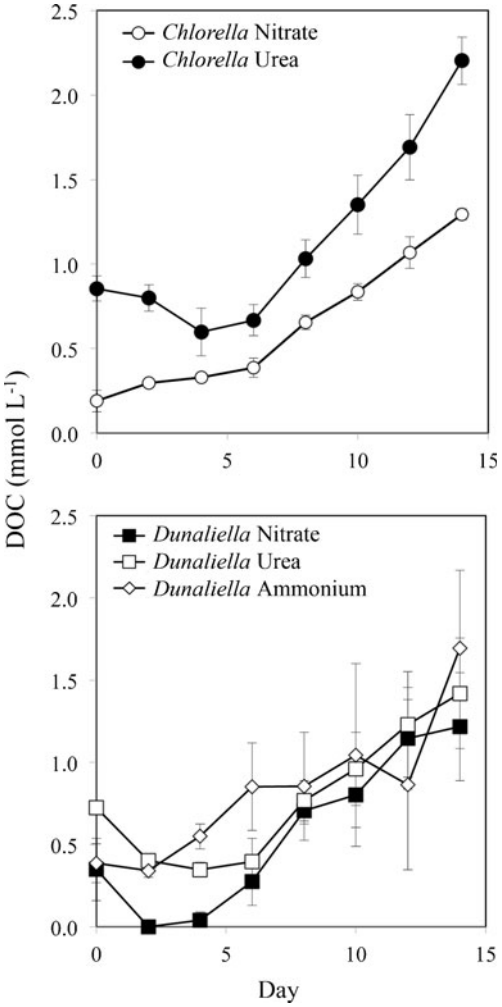

Fig. 2 The concentration of DOC (millimoles per litre) in 20-L photobioreactor cultivations of *C. vulgaris* (nitrate, urea) and *D. tertiolecta* (nitrate, urea, ammonium). Data points are mean of three replicates; *error bars* are standard deviations

the experiments, bacterial cells numbered 1.1×10^{11} (SD $\pm3.2\times10^{10}$) cells L^{-1} in the ammonium treatment, compared to 2.4×10^{10} (SD $\pm4.0\times10^{9}$) and 2.2×10^{10} (SD $\pm7.6\times10^{9}$) cells L^{-1} in the urea and nitrate treatments. Bacteria may only be a small fraction of the total biomass present in healthy algal cultures but are critically important for recycling organic nitrogen compounds to biologically utilisable forms, as shown by the nitrite and ammonium dynamics in the systems used here (Fig. 4).

D. tertiolecta Accessory Measurements

Changes in the maximum quantum yield of photosystem II (F_v/F_m) during cultivation of *D. tertiolecta* are shown in Fig. 1a. Initial values ranged from 0.70 (SD ±0.01) to 0.75 (SD ±0.03) indicating healthy, efficient photosynthetic performance [55]. F_v/F_m values declined with time, reaching 0.44 (0.06) to 0.57 (0.04) by the end of the cultivations. $F_v/$

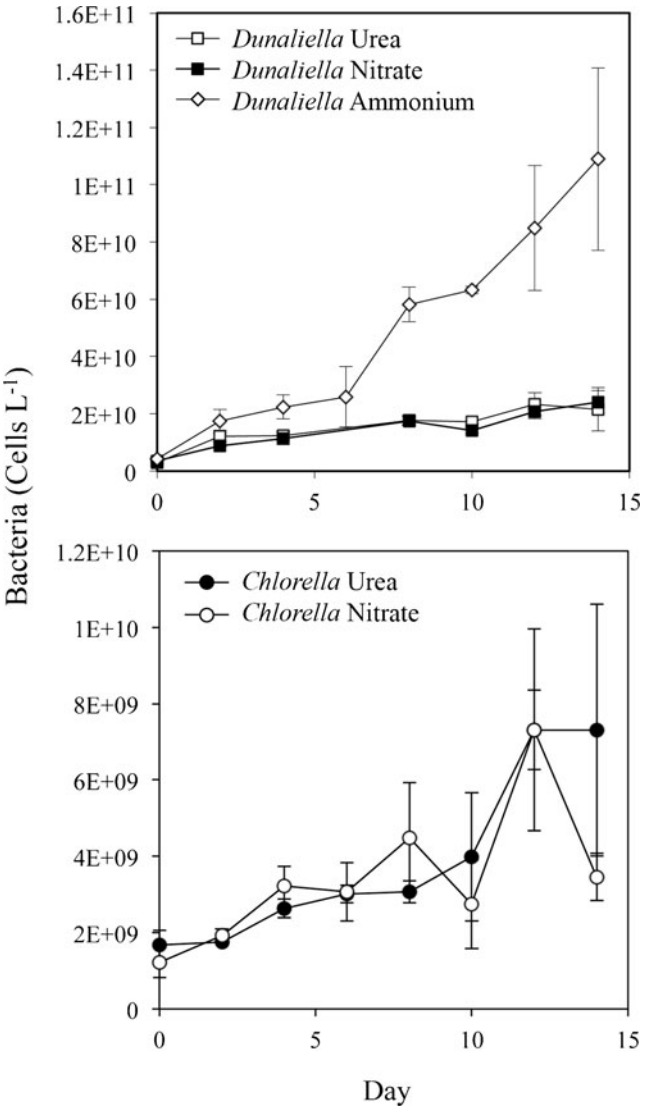

Fig. 3 Bacterial cell number (cells per litre) in cultivations of *C. vulgaris* and *D. tertiolecta* supplied with alternative nitrogen sources. Data points are mean of three replicates; error bars are standard deviations

F_m values for cultivations supplied with ammonium were lower than the nitrate and urea treatments (Fig 1a).

Changes in the pH of *D. tertiolecta* treatments were minimal due to the relatively high buffering capacity of the saline media. Cultivation using ammonium leads to a slight acidification (mean ΔpH −0.27), whilst cultivation using urea and nitrate leads to an increase in mean pH of 0.68 and 0.73, respectively (Fig.1a).

Dissolved oxygen concentrations in 20-L photobioreactors containing either species remained <20% above air saturation throughout cultivation (Fig. 1a), indicating little impact on cultivation performance. These values are a result of efficient removal due to the high sparging rate employed.

C. vulgaris Accessory Measurements

The F_v/F_m dynamics in cultivations of *C. vulgaris* followed a similar trend to those of *D. tertiolecta* (Fig. 1b). F_v/F_m declined whilst growth in biomass remained relatively constant, and it should be noted that the maximum quantum yield of charge separation in photosystem II and CO_2 fixation/growth are not necessarily tightly coupled. However, F_v/F_m provides a useful measure of physiological stress during batch cultivation [55]. Changes in the cultivation pH are shown in Fig.1b, including the *C. vulgaris* ammonium treatment. Cultivation of *C. vulgaris* using urea caused an increase in mean pH of 0.65, whilst cultivation using nitrate led to an increase of 3.05. The pH of microalgal cultivations is modified (a) by uptake of inorganic carbon from the medium (increases pH), (b) nitrification in ammonium treatments and (c) by microalgal uptake of nitrogen compounds. Uptake of nitrogen compounds follows charge balance: Ammonium uptake leads to H^+ production (decreases pH), whilst uptake of NO_3^- produces OH^- (increases pH). Since urea uptake should not affect the pH significantly [25], the increase in pH in urea treatments can be attributed to the depletion of dissolved inorganic carbon. The pH increase in nitrate treatments was higher than the equivalent urea treatments and could be attributed to the additive effect of nitrate uptake plus inorganic carbon depletion.

The rapid acidification observed in the *C. vulgaris* ammonium treatment was attributed to NH_4^+ uptake by microalgae, bacterial nitrification and the low buffering capacity of the freshwater media. Bacterial nitrification may be an important process when using ammonium as a nitrogen source [42] and is concordant with the strong bacterial growth observed (Fig. 2). Buffering solutions or acid/base addition may be used to stabilise the pH of microalgal cultures [71]. However, this is unlikely to be practical for large-scale algal production involving considerable volumes of liquid medium, together with additional cost, and was not considered an appropriate avenue for further investigation. Cultivating freshwater species to high cell density using ammonium as the sole nitrogen source may thus be impractical for commercial production.

Bubble Columns

Growth and Nitrogen Uptake in Bubble Columns

Growth in dry weight of *C. vulgaris* and *D. tertiolecta* cultivated in bubble columns using nitrate as the nitrogen source is shown in Fig. 4. Treatments using simulated flue gas (12% CO_2) grew rapidly, at maximum rates of 0.60 (SD ±0.11) and 0.83 (SD±0.13)g L^{-1} day^{-1} for *C. vulgaris* (days 4 to 6) and *D. tertiolecta* (days 2 to 4), respectively

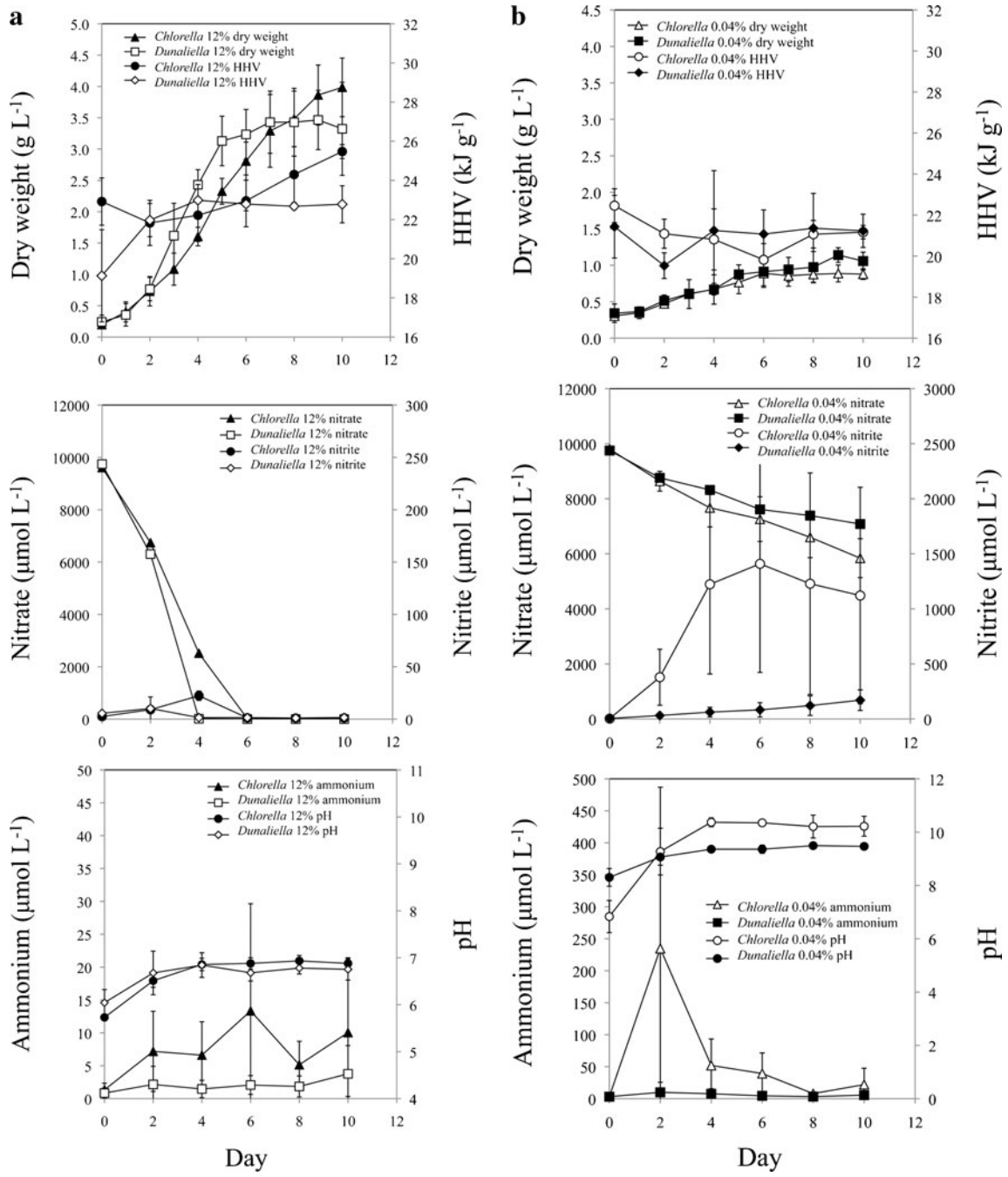

Fig. 4 **a** Growth performance of cultivations of *D. tertiolecta* and *C. vulgaris* in bubble-column experiments using simulated flue gas (12%). Data points are mean (n=3); *error bars* are standard deviations. **b** Growth performance of cultivations of *D. tertiolecta* and *C. vulgaris* in bubble-column experiments using air CO_2 concentrations (0.04%). Data points are mean (n=3); *error bars* are standard deviations

(Fig. 4a). Treatments cultivated in 0.04% CO_2 were carbon-limited and never reached the carrying capacity of the available nitrogen and phosphorus (Fig. 4b). Growth rates in air were 0.10 (SD±0.04) and 0.07 (SD±0.02) g L^{-1} day^{-1} for *C. vulgaris* and *D. tertiolecta*, respectively.

D. tertiolecta and *C. vulgaris* cultivated in 12% CO_2 had mean nitrogen uptake rates of 44.0 (SD ±11.1) and 17.6 (SD ±12.3) mg N L^{-1} day^{-1} during the period of maximum growth, and >99.9% of the available nitrogen was removed between days 4 and 6 (Fig. 4a). Cultivations of *D. tertiolecta* and *C. vulgaris* using 0.04% CO_2 assimilated inorganic nitrogen from the medium at a rate of 2.9 (SD ±2.4) and 2.0 (SD ±2.2) mg N L^{-1} day^{-1}. The higher heating value of *C. vulgaris* cultivated using 12% CO_2 increased in response

to nitrogen limitation, but as in the 20-L photobioreactor experiments, the effect on *D. tertiolecta* was negligible (Fig. 4a).

Dissolved Organic Carbon and Dissolved Organic Nitrogen

The concentration of DOC increased over the batch cycle, reaching 17.19 (SD±4.37) and 6.03 (SD±2.49) mmol L^{-1} at the end of *D. tertiolecta* and *C. vulgaris* cultivations, respectively. At day 10, the concentration of DON reached 0.25 (SD±0.04) and 0.33 (SD±0.08) mmol L^{-1} for the same species, corresponding to mean C/N ratios of 69:1 and 18:1. The DOC/DON ratio of dissolved organic matter in 12% CO_2 cultivations of *C. vulgaris* at day 10 was approximately the same as the corresponding cellular C/N ratio at the same point. In contrast, the dissolved substances exuded by *D. tertiolecta* at the same point had a DOC/DON almost 5-fold higher than the cellular C/N ratio (14:1). An important difference between species is that *C. vulgaris* possesses a cell wall structure, whilst *D. tertiolecta* does not [53]. This physical difference may be the cause of higher concentrations of DOC released by *D. tertiolecta* and may explain the differences in C/N ratio of dissolved substances exuded by either species. The difference in C/N ratio of DOM may also be due to differences in the relative utilisation of DOC and DON by the bacteria in saline and freshwater media.

Nitrite and Ammonium Dynamics

The presence of bacteria ("Dissolved Organic Carbon and Bacterial Cell Number") and release of DON from cells suggest that a fraction of the nitrogen supplied is cycled within the cultivation system. Both nitrite and ammonium are products of bacterial metabolism of organic nitrogenous substrates, and Fig. 4 shows the concentration of each of these compounds during cultivation in bubble columns. Bacteria may compete with microalgae for nitrogen, assimilating a fraction whilst recycling algal-derived organic nitrogen to forms that can be easily utilised by photoautotrophs [57]. However, the implications of bacterial activity for applied microalgal cultivations has received little research to date and clearly is an area for further experimentation.

Industrial-Scale Nitrogen Consumption and Energy Demands

How Important Is the Nitrogen Energy Burden?

One of the main questions arising from life-cycle assessments concerns identifying the dominant energy burdens involved in microalgal cultivation, thereby identifying which components should be targeted for improvement. The relationship between different aspects of microalgal cultivation is complex and case-specific [2, 4, 14, 35, 36, 39, 45, 56, 69]. The energy embedded in the supply of nitrogen has been suggested as the major variable determining the sustainability of microalgal technologies [14, 45]. However, others have suggested it is the supply of power for cultivation mixing that is the dominant burden [39, 69]. However, the most recent assessments identify significant issues with supply of nutrients and CO_2 to algal cultivation systems. Beal et al. [4] showed that without discounted energy inputs (e.g. recycling nutrients, carbon), the energy return on the energy invested was below unity. Similarly, Pate et al. [56] identify that provision of nutrients, CO_2 and also water are the greatest constraints on the ability to sustainably produce microalgal biomass on a large scale. The following section examines the relative importance of power input, fertiliser requirement and CO_2 on the energy efficiency of the intensive bubble-column reactors operated using 12% CO_2.

Energetic Performance of Bubble-Column Photobioreactors Using 12% CO_2

The gross energy production (kilojoules per litre) during batch cultivation of *C. vulgaris* and *D. tertiolecta* was calculated as the product of the microalgal dry weight and HHV at each point during growth (Eq. 1).

$$e_B = b \times h \tag{1}$$

where e_B is the energy contained in biomass of 1 L of bioreactor fluid (kilojoules per litre), b is the biomass dry weight (grams per litre), h is the higher heating value (kilojoules per gram). The power input/energy demand for pumping gas through the photobioreactor was calculated according to Sierra et al. [67] (Eqs. 2 and 3). Note that two scenarios for power supply are shown. The 50-W m^{-3} scenario (corresponds to a daily energy input of 4.2 and 4.3 kJ L^{-1} day^{-1} for fresh and saline media, respectively) is that actually used in the research, presented and typical for such photobioreactor systems [62]. The second (theoretic) scenario corresponds to an alternative where the power supply was minimised to a daily average of 15 W m^{-3} (corresponds to a daily energy input of 1.2 to 1.3 kJ L^{-1} day^{-1} for fresh and saline media, respectively), with the assumption of no significant impact on productivity.

$$P_G/V_L = \rho_L \times g \times U_{sg} \tag{2}$$

$$U_{sg} = V_G/A \tag{3}$$

where P_G/V_L is the power supplied per unit volume (Watts per cubic metre), ρ_L is the liquid density (kilograms per cubic metre), g is the rate of gravitational acceleration (9.8 m^2 s^{-1}), U_{sg} is the superficial gas velocity (0.005 m s^{-1}),

V_G is the gas flow rate (cubic metres per second) and A is the cross-sectional area of the bubble column (square metres). The energy embedded in supply of nitrogen fertiliser was derived from the product of the mass of fertiliser nitrogen removed from the culture fluid and the energy demand for production of nitrogen fertiliser (50 kJ g^{-1}, Table 3) (Eq. 4).

$$e_{TN} = m_N \times e_N \quad (4)$$

where e_{TN} is the total energy embedded in supply of nitrogen at each point during growth (kilojoules per litre), m_N is the mass of nitrogen removed from the fluid at each point of the growth cycle (grams per litre), e_N is the energy embedded in each unit mass of nitrogen (50 kJ g^{-1}; Table 4). The mass of phosphorus fertiliser consumed was calculated using the mass of fertiliser nitrogen removed from the culture fluid and the Redfield stoichiometry (N/P, 16:1 mol, 7.23:1 mass), by dividing the mass of elemental nitrogen consumed by 7.23. The mass of phosphorus consumed was then multiplied by 44 kJ g^{-1} [4] to derive the energy embedded in supply of phosphorus.

$$e_{TP} = (m_N/7.23) \times e_P \quad (5)$$

where e_{TP} is the energy embedded in supply of phosphorus at each point of the growth curve (kilojoules per litre), m_N is the mass of nitrogen consumed at each point during growth (grams per litre), 7.23 is the Redfield mass ratio (N/P) and e_P is the energy embedded in a unit mass of phosphorus fertiliser (44 kJ g^{-1} P). The amount of energy embedded in supply of CO_2 (assuming flue gas was not available and stored CO_2 was supplied) was calculated by multiplying the amount of CO_2 supplied daily (20.7 g day^{-1}, Table 1) by 7.33 kJ g^{-1} [4].

$$e_{TCO_2} = m_{CO_2} \times e_{CO_2} \quad (6)$$

where e_{TCO_2} is the energy embedded in supply of CO_2 to cultivations at each point of the growth cycle (kilojoules per litre), m_{CO_2} is the mass of CO_2 supplied at each point (grams CO_2 per day, Table 1 × days) and e_{CO_2} is the energy embedded in supply CO_2 from manufactured sources (non-flue gas, 7.33 kJ g^{-1}). The effect of cumulative subtraction of each of these energy burdens on the net bioenergy production during growth in bubble-column photobioreactors is shown in Fig. 5. Both the actual (kilojoules per litre) and net energy ratios [39] are shown. Inset figures include the CO_2 burden due to extreme negative net energy balance.

At day 10, the supply of nitrogen to *C. vulgaris* and *D. tertiolecta* reduced the gross energy production by 7% and 10%. However, in this reactor configuration, the supply of power far outweighed the impact of nutrient energy consumption. At day 4 of *D. tertiolecta* cultivation, consumption of fertiliser nitrogen accounted for a peak of 27% of both energy burdens combined. When nitrogen was exhausted from the media (after 4–6 days), power became even more dominant and accounted for 86% of the energy consumed in production in both species cultivations at day 10. It is possible to operate gas-sparged reactors using power inputs lower than 50 W m^{-3} [35, 62, 77]. As an example, a power input of 15 W m^{-3} (as opposed to the 50 W m^{-3}) would cause the fertiliser energy demand to equal the power demand during nutrient-replete growth (days 2–6) but drop to 50% of the power demand by day 10 (Table 4). This calculation assumes the same gross energy production is possible at low power input, although in practice it may be lower [35]. Accounting for the nitrogen-embedded energy and power (50 W m^{-3}) burdens only, NER >1 was achieved in cultivation of both species. For *C. vulgaris*, a peak NER of 1.85 was achieved at day 10, whilst for *D. tertiolecta* a peak NER of 2.16 was achieved at day 4 (Fig. 5).

The energy embedded in the supply of phosphorus was an order of magnitude lower than supply of nitrogen in these calculations. However, of particular importance is the supply of CO_2 (Fig. 5). It should be noted that the photobioreactor configuration tested here was not optimised to minimise CO_2 inputs; however, the scale of the CO_2 energy debt is such that even if 1% CO_2 (as opposed to 12% used here) were supplied at a flow rate equivalent to 15 W m^{-3}, with no loss of productivity and very efficient CO_2 mass transfer, the net energy return (kilojoules per litre) would barely rise above zero even at the optimal part of the growth curve. It is thus essential to utilise concentrated waste CO_2 (e.g. from power plant) in order to avoid the significant energy burden that would otherwise be incurred by supply from purified, compressed sources to gas-sparged photobioreactor systems.

Table 4 Fossil energy required to produce 1 kg of fertiliser nitrogen (as elemental nitrogen, megajoules per kilogram of N) as reported for various fertiliser types found in the literature

Reference	MJ kg^{-1} nitrogen	End product
[1]	35	Ammonium nitrate[a]
[1]	2.3–3.7	Ammonium nitrate[b]
[61]	Approximately 40–50	Ammonia, urea, ammonium nitrate, calcium ammonium nitrate[c]
[16]	43–78 (norm 50)	Nitrogen[d]
[46]	50	Nitrogen[e]
[74]	41	Ammonium nitrate[f]
[74]	49	Urea[f]
[74]	43	Ammonium sulphate[f]
[4]	59	Urea

[a] Natural gas plant, modelled for fossil energy

[b] Biogas plant, modelled for fossil energy

[c] Based on most recent data (2000–on)

[d] Not including diesel contribution to application, all references pre-1991

[e] Cumulative primary energy consumption

[f] Includes burdens of producing packing and delivering

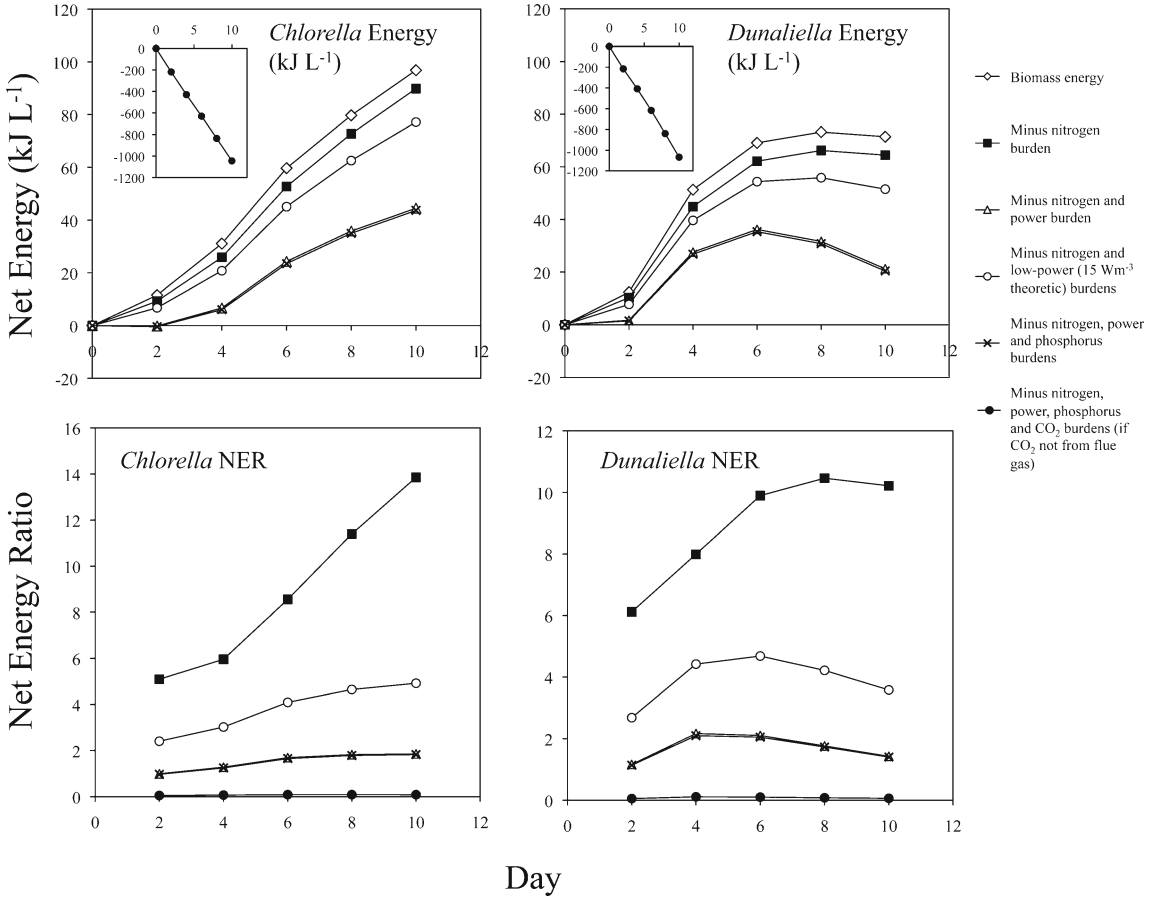

Fig. 5 Energetic performance of *C. vulgaris* and *D. tertiolecta* cultivated in bubble-column photobioreactors supplied with 12% CO_2 over the batch production cycle showing both net energy (kilojoules per litre) and net energy ratio. Calculation of respective energy burdens is as described in "Energetic Performance of Bubble-Column Photobioreactors Using 12% CO_2"

Optimal Harvest Point

The optimal harvest point needs to be established, in order to minimise the requirement for fertiliser inputs and energy into microalgal production systems. Clearly the nitrogen utilisation efficiency is important, but so too is its impact on growth rate, photosynthetic efficiency (PE), lipid content and calorific value. The relative impacts of each of the parameters are presented in Table 5. The impacts of nitrogen limitation on photosynthetic efficiency are not well described within the context of microalgal biomass production. This is important because proponents of microalgal technologies often cite high PE as the key reason for development. However, any compromises on PE, such as nitrogen fertiliser deprivation [45], immediately reduce the competitiveness of microalgal mass culture with terrestrial crops.

Table 5 Relative advantages (+) and disadvantages (−) of batch and continuous production processes on performance of microalgal cultivations

	Continuous production	Batch Production
Nitrogen utilisation efficiency	−	+
Heating value	−	+
Lipid content	−	+
Productivity	+	−
Photosynthetic efficiency	+	−
Power input per unit biomass	+	−

The term 'batch' refers to a nitrogen-limited end phase, whilst 'continuous' describes nutrient-replete growth

Industrial CO_2 Mitigation Perspectives on Nitrogen Fertiliser Demands

In order to convert 1 tonne of CO_2 to organic matter, it is necessary to produce approximately 0.55 tonnes of microalgal biomass (microalgal biomass contains approximately 50% carbon). For nutrient-replete microalgal biomass, the Redfield C/N ratio of 5.7:1 (mass) indicates that 0.10 tonnes of nitrogen (elemental nitrogen) is required per tonne of

CO_2 mitigated [24, 40]. In 2000, global CO_2 emissions from coal-fired power plant alone measured 8 Gt $year^{-1}$ [22]. Thus, uptake of 10% of this CO_2 would require supply of 80 Mt of nitrogen fertiliser annually, equivalent to current global production [61]. However, the benefit of using algae to remove CO_2 and produce a fuel is actually derived from using the biomass energy to displace fossil fuels that would otherwise be combusted [7]. As a result, the environmental benefits of CO_2 mitigation and bioenergy production using microalgae are only equivalent to the net life-cycle calorific value and CO_2 fossil-equivalent emissions savings. This means that to mitigate 10% of the global CO_2 emissions, on a net life-cycle basis, would actually require production of significantly more algal biomass and use more nitrogen fertilisers than predicted above. Unfortunately, many life-cycle assessments have shown that the life-cycle energy return for many potential microalgal projects using current technology would be <1 [2, 4, 14, 39, 45, 56, 69], so that the energetic costs involved in production of microalgal biofuels would lead to emission of considerably more CO_2 than would be saved by displacing some fossil fuels. In this work, we only considered the major energy burdens associated with biomass production. The maximum net energy return was 1.82 and 2.10 for *C. vulgaris* and *D. tertiolecta* considering power, nitrogen and phosphorus energy inputs. If concentrated CO_2 could not be sourced locally (e.g. flue gas from power plant), the NER of biomass production including provision of manufactured CO_2 would decrease to 0.09 and 0.11 for *C. vulgaris* and *D. tertiolecta*. In this situation, microalgal biomass production using this type of photobioreactor configuration would be unsustainable. Further, there are other impacts not considered in this work which may also reduce the energetic efficiency of these systems. These include supply of suitable water, trace nutrients, photobioreactor materials and additional efficiency losses associated with supply of power [4, 35, 45].

However, there are a number of technologies available that may improve process efficiency. Nutrients and energy may be re-cycled by anaerobic bioprocesses [10, 17, 44, 66], via residues from thermochemical conversion processes such as pyrolysis and gasification [6, 28]. Nutrients may also be supplied via wastewater streams [14, 50, 70]. However, use of wastewater streams may present problems with security of supply. Additional technologies may include optimisation of power input [35].

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References

1. Ahlgren S, Bernesson S, Nordberg A, Hansson P (2010) Nitrogen fertiliser production based on biogas—energy input, environmental impact and land use. Bioresour Technol 101:7181–7184
2. Batan L, Quinn J, Willson B, Bradley T (2010) Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae. Environ Sci Technol 44:7975–7980
3. Bauen A (2006) Future energy sources and systems—acting on climate change and energy security. J Power Sources 2:893–901
4. Beal CM, Hebner RE, Webber MS, Ruoff RS, Seibert AF (2011) The energy return on investment for algal biocrude: results for a research production facility. BioEnerg Res. doi:10.1007/s12155-011-9128-4
5. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian J Physiol Pharm 37:911–917
6. Brown TM, Duan P, Savage PE (2010) Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. Energ Fuel 24:3639–3646
7. Campbell PK, Beer T, Batten D (2011) Life cycle assessment of biodiesel production from microalgae in ponds. Bioresour Technol 102:50–56
8. Carballo-Cardenas EC, Tuan PM, Janssen M, Wijffels RH (2003) Vitamin E (alpha-tocopherol) production by the marine microalga *Dunaliella tertiolecta* and *Tetraselmis suecica* in batch cultivation. Biomolecular Eng 20:139–147
9. Carlson CA (2002) Production and removal processes. In: Carlson CA, Hansell D (eds) Biogeochemistry of marine dissolved organic matter. Academic, London, pp 91–139
10. Carver SM, Hulatt CJ, Thomas DN, Tuovinen OH (2010) Thermophilic, anaerobic co-digestion of microalgal biomass and cellulose for H_2 production. Biodegradation. doi:10.1007/s10532-010-9419-z
11. Chen M, Tang H, Ma H, Holland T, Ng K, Salley S (2011) Effects of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresour Technol 102:1649–1655
12. Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306
13. Chiu SY, Kao CY, Chen CH, Kuan TC, Ong SC, Lin CS (2008) Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresour Technol 99:3389–3396
14. Clarens A, Resurrecion E, White M, Colosi M (2010) Environmental life cycle comparison of algae to other bioenergy feedstocks. Environ Sci Technol 44:1813–1819
15. Dalgaard T, Halberg N, Porter J (2001) A model for fossil energy use in Danish agriculture used to compare organic and conventional farming. Agr Ecosyst Environ 87:51–65
16. Davis R, Aden A, Pienkos P (2011) Techno-economic analysis of autotrophic microalgae for fuel production. Appl Energ 88:3524–3531
17. De Schamphelaire L, Verstraete W (2009) Revival of the biological sunlight-to-biogas energy conversion system. Biotechnol Bioeng 103:296–304
18. Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC (2008) Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 19:235–240
19. Douskova I, Doucha J, Livansky K, Machat J, Novak P, Umysova D et al (2009) Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Environ Biotechnol 82:179–185

20. Fabregas J, Abalde J, Herrerco C (1989) Biochemical composition and growth of the marine microalga *Dunaliella tertiolecta* (Butcher) with different ammonium nitrogen concentrations as chloride, sulphate, nitrate and carbonate. Aquaculture 83:289–304
21. Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P (2008) Land clearing and the biofuel carbon debt. Science 319:1235–1238
22. Feron PHM (2009) The potential for the improvement of the energy performance of pulverised coal fired power stations with post-combustion capture of carbon dioxide. Energy Procedia 1:1067–1074
23. Friedl A, Padouvas E, Rotter H, Varmuza K (2005) Prediction of heating values of biomass fuel from elemental composition. Anal Chim Act 544:191–198
24. Geider RJ, Roche JL (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17
25. Goldman JC, Brewer PG (1980) Effect of nitrogen source and growth rate on phytoplankton-mediated changes in alkalinity. Limnol Oceanogr 25:352–357
26. Gordillo FJL, Goutx M, Figueroa FL, Niell FX (1998) Effects of light intensity, CO_2 and nitrogen supply on lipid class composition of *Dunaliella viridis*. J Appl Phycol 10:135–144
27. Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis, 2nd edn. Chemie, Wienheim
28. Grierson S, Strezov V, Ellem G, McGregor R, Herbertson J (2009) Thermal characterization of microalgae under slow pyrolysis conditions. J Anal Appl Pyrol 85:118–123
29. Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21:493–507
30. Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006) Environmental, economic, and energetic costs and benefits of biodiesel and ethanol fuels. P Natl Acad Sci-Biol 25:1206–11210
31. Holmes RM, Aminot R, Kerouel B, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine and fresh water. Can J Fish Aquat Sci 56:1801–1808
32. Hsieh CH, Wu WT (2009) Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresour Technol 100:3921–3926
33. Hughes E (2000) Biomass cofiring: economics, policy and opportunities. Biomass Bioenerg 19:457–465
34. Hulatt CJ, Thomas DN (2010) Dissolved organic matter (DOM) in microalgal photobioreactors: a potential loss in solar energy conversion? Bioresour Technol 101:8690–8697
35. Hulatt CJ, Thomas DN (2011) Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. Bioresour Technol 102:5775–5787
36. Hulatt CJ, Thomas DN (2011) Energy efficiency of an outdoor microalgal photobioreactor sited at mid-temperate latitude. Bioresour Technol 102:6687–6695
37. Hu H, Gao K (2006) Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnol Lett 28:987–992
38. Hu Q, Sommerfeld M, Jarvis E, Ghihardi M, Posewitz M, Seibert M et al (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639
39. Jorquera O, Kiperstock A, Sales EA, Embirucu M, Ghirardi ML (2010) Comparative energy life-cycle analysis of microalgal biomass production in open ponds and photobioreactors. Bioresour Technol 101:1406–1413
40. Klausmeier CA, Litchman E, Daufresne T, Levin SA (2008) Phytoplankton stoichiometry. Ecol Res 23:479–485
41. Kroon H (1993) Determination of nitrogen in water: comparison of continuous flow method with on-line UV digestion with the original Kjedahl method. Anal Chim Acta 276:287–293
42. Kubo Y, Takasu F, Shimura R, Nagaoka S, Shigesada N (1999) Population dynamics of nitrifying bacteria in an aquatic ecosystem. Biol Sci Space 13:333–340
43. Lakaniemi AM, Intihar VM, Tuovinen OH, Puhakka JA (2012) Growth of *Chlorella vulgaris* and associated bacteria in photobioreactors. Microb Biotechnol. 5:69–78
44. Lakaniemi AM, Hulatt CJ, Thomas DN, Puhakka JA (2011) Biogenic hydrogen and methane production from *Chlorella vulgaris* and *Dunaliella tertiolecta* biomass. Biotechnol Biofuels 4:34. doi:10.1186/1754-6834-4-34
45. Lardon L, Helias A, Sialve B, Steyer JP, Bernard O (2009) Life-cycle assessment of biodiesel production from microalgae. Environ Sci Technol 43:6475–6481
46. Lewandowski I, Schmidt U (2006) Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agr Ecosyst Environ 112:335–346
47. Lombardi AT, Wangersky PJ (1995) Particulate lipid class composition of three marine phytoplankters *Chaetoceros gracilis*, *Isochrysis galbana* (Tahiti) and *Dunaliella tertiolecta* grown in batch culture. Hydrobiologia 306:1–6
48. Lopez CVG, Garcia MCC, Fernandez FGA, Bustos CS, Chisti Y, Sevilla JMF (2010) Protein measurements of microalgal and cyanobacterial biomass. Bioresour Technol 101:7587–7591
49. Lourenco SO, Barbarino E, Mancini-Filho J, Schinke KP, Aidar E (2002) Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation for aquaculture. Phycologia 41:158–168
50. Lundquist TJ, Woertz IC, Quinn NWT, Benemann JR (2010) A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, Berkeley, pp 1–178
51. Mandalam RK, Palsson B (1998) Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures. Biotechnol Bioeng 59:605–611
52. Mulvenna PF, Savidge G (1992) A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent. Estuar Coast Shelf Sci 34:429–438
53. Oliveira L, Bisalputra T, Antia N (1980) Ultrastructural observation of the surface coat of *Dunaliella tertiolecta* from staining with cationic dyes and enzyme treatments. New Phytol 85:385–392
54. Pahl SL, Lewis DM, King KD, Chen F (2011) Heterotrophic growth and nutritional aspects of the diatom *Cyclotella cryptica* (Bacillariophyceae): effect of nitrogen source and concentration. Bioresour Technol. doi:10.1007/s10811-011-9680-5
55. Parkhill JP, Maillet G, Cullen JJ (2001) Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J Phycol 37:517–529
56. Pate R, Klise G, Wu B (2011) Resource demand implications for US algae biofuels production scale-up. Applied Energy 88:3377–3388
57. Patil SS, Kumar MS, Ball AS (2010) Microbial community dynamics in anaerobic bioreactors and algal tanks treating piggery wastewater. Appl Microbiol Biotechnol 87:353–363
58. Perez-Garcia O, Escalante FME, De-Bashan LE, Bashan Y (2010) Heterotrophic cultures of microalgae: metabolism and potential products. Water Res. doi:10.1016/j.watres.2010.08.037
59. Pick U, Weiss M (1991) Polyphosphate hydrolysis within acidic vacuoles in response to amine-induced alkaline stress in the halotolerant alga *Dunaliella salina*. Plant Physiol 97:1234–1240
60. Qian J, Mopper K (1996) An automated, high performance, high temperature combustion dissolved organic carbon analyzer. Anal Chem 68:3090–3097
61. Ramirez CA, Worrell E (2006) Feeding fossil fuels to the soil: an analysis of energy embedded in technological learning in the fertilizer industry. Resour Conserv Recy 46:75–93

62. Reyna-Velarde R, Cristiani-Urbina E, Hernandez-Melchor DJ, Talasso F, Canizares-Villanueva RO (2010) Hydrodynamic and mass transfer characterization of a flat-panel airlift photobioreactor with high light path. Chem Eng Process 49:97–103
63. Sanchez OJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 99:5270–5295
64. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnung JH, Posten C et al (2008) Second generation biofuels: high efficiency microalgae for biodiesel production. Bioenerg Res 1:20–43
65. Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's aquatic species program —biodiesel from algae. National Renewable Energy Laboratory, Golden
66. Sialve B, Bernet N, Bernard O (2009) Anaerobic digestion as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 27:409–416
67. Sierra E, Acien FG, Fernandez JM, Garcia JL, Gonzalez C, Molina E (2008) Characterization of a flat plate photobioreactor for the production of microalgae. Chem Eng J 138:136–147
68. Soletto D, Binaghi L, Lodi A, Carvalho JCM, Converti A (2005) Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. Aquaculture 243:217–224
69. Stephenson AL, Kazamia E, Dennis JS, Howes CJ, Scott SA, Smith AG (2010) Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors. Energ Fuel 24:4062–4077
70. Sturm BSM, Lamer SL (2011) An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl Energ 88:3499–3506
71. Tam NFY, Wong YS (1996) Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media. Bioresour Technol 57:45–50
72. Vunjak-Novakovic G, Kim Y, Wu X, Berzin I, Merchuk JC (2005) Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot plant studies. Ind Eng Chem Res 44:6154–6163
73. Weyer KM, Bush DR, Darzins A, Willson BD (2009) Theoretical maximum algal oil production. Bioenerg Res 2:204–213
74. Williams AG, Audsley E, Sandars D (2010) Environmental burdens of producing bread wheat, oilseed rape and potatoes in England and Wales using simulation and system modelling. Int J Life Cycle Assess 15:855–868
75. Xin L, Hong-Ying H, Ying-Xue S (2010) Effects of different nitrogen and phosphorus concentrations on the nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. Bioresour Technol 101:5494–5500
76. Xu L, Brilman DWF, Withag JAM, Brem G, Kersten S (2011) Assessment of a wet and dry route for the production of biofuels from microalgae: energy balance analysis. Bioresour Technol 102:5113–5122
77. Zhang K, Miyachi S, Kurano N (2001) Photosynthetic performance of a cyanobacterium in a vertical flat-plate photobioreactor for outdoor microalgal production and fixation of CO_2. Biotechnol Lett 23:21–26