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Nutrients from anaerobic digestion effluents for cultivation of the microalga *Nannochloropsis* sp. – impact on growth, biochemical composition and the potential for cost and environmental impact savings

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Abstract

Microalgal biotechnology has yielded a range of products for different consumer markets, but large scale production for bulk commodities is limited by the cost and environmental impact of production. Nutrient requirements for large-scale production contribute significantly to the cost and environmental impact of microalgal biomass production and should subsequently be addressed by more careful sourcing of nutrients. This study assessed the use of nitrogen and phosphorus contained in effluents from anaerobic digestion of food waste to cultivate the marine microalga *Nannochloropsis* sp.. With suitable dilution, effluent could replace 100% of nitrogen demands and 16% of required phosphorus, without significant impacts on growth or biomass productivity. Additional phosphorus requirements could be decreased by increasing the N:P molar ratio of the media from 16:1 to 32:1. *Nannochloropsis* sp. accumulated lipid up to 50% of dry weight under N- stress, with significant increases in the content of saturated and mono-unsaturated fatty acids. Using empirical data generated in this study, the cost and environmental impact of nitrogen and phosphorus supply was assessed versus the use of fertilizers for biomass and biodiesel production. Nutrient requirements predicted by the Redfield Ratio overestimating impacts by as much as 140% compared to empirical data. By utilising residual nutrients and optimising nutrient supply, the cost and environmental impact of nitrogen and phosphorus were decreased by 90% versus the use of artificial fertilizers.

Key words: microalgae, anaerobic digestate effluents, nutrient sustainability, biomass, biodiesel, LCA

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1.   Introduction

Microalgae are considered an important feedstock for the production of a range of bulk and fine chemicals with applications in markets spanning food, feeds and cosmetics, as well as their much touted bioenergy potential [1,2]. However, expansion into production of bulk compounds with lower market values is limited, not least by profitability [3], but also the inherent energy requirements, greenhouse gas (GHG) emissions and other resource intensive aspects of production [4–7]. An approach to decrease the cost and environmental footprint of production is the integration of production with industrial infrastructure to make use of cheap inputs such as locally regenerated inorganic nutrients, CO_2, water and heat, possibly forming a more sustainable biorefinery [8].

Nutrient inputs for microalgal cultivation have a considerable impact on the economics and environmental sustainability of biomass production if high purity fertilizers are used [8,9] or if nutrients are not recycled effectively in these processes [6,10]. It has been estimated that fertilizer production can contribute between 10–60% of the cumulative energy required for biomass production [5,7,11–13]. The production of nitrogen (N) based fertilizers is heavily dependent on natural gas resources [14], subsequently the CO_2-equivalent (CO_2-eq) footprint of ammonia synthesis, the base of many N-fertilizers, is over 2 kg CO_2-eq produced per kg N [15]. If bioenergy production is the target of microalgal cultivation, then reliance on fossil reserves to produce fertilizers could result in significant problems in the future as demand increases and fossil reserves begin to wane [16], likely increasing the cost of N fertilizer production.

It is expected that due to the fertilizer requirements of large scale microalgal production, significant impacts will be felt ~~on~~ by other markets, in particular, agricultural food production. This is especially critical in the case of P, which is derived from mineral extraction and is anticipated to become a limiting resource for agricultural and industrial sectors by the end of the century [17]. The U.S. Energy Independence and Security Act's (signed 2007) has set a target of producing 79 billion L of advanced biofuels per year by 2022 to aid in decreasing reliance on energy imports and aid establishing new sustainable energy targets. Canter et al. [6] calculated that to meet 24% of this target with microalgae derived fuel (19 billion L yr^{-1}), biomass production would consume 26–28% and 15–

23% of current U.S. usage of N and P (-P_2O_5) fertilizer, respectively [6]. These requirements are on par with other large-scale agricultural requirements, such as for corn and soybean production [18], and could lead to increased fertilizer prices and decreased incentive for microalgal biofuel production. It is hence critical to develop strategies for efficient nutrient utilisation for large scale microalgal cultivation.

Research to decrease the use of costly fertilizer based nutrients take three main approaches: 1) optimisation of nutrient supply to avoid wastage [19,20], 2) establishing the use of more sustainable sources of nutrients, potentially from waste streams [21], and 3) the recycling of nutrients within the microalgal biorefineries themselves [6,22]. The use of nutrients from waste streams has received considerable attention for biofuel production from microalgae, but the cultivation of microalgal biomass for feed or food applications may be restricted by the source of nutrients that can be used. In particular, concerns may be raised over the presence of pathogens and high concentrations of metals or toxic compounds in the waste streams [23–25]. Some of these issues are discussed in the review of van der Spiegel et al. [24], but it is clear that there are still uncertainties in the regulation of waste nutrient usage in microalgal cultivation and these risks require further assessment and eventually suitable legislation.

A nutrient source that may avoid some of the issues associated with using municipal sewage or industrial wastes for biomass production are anaerobic digestion (AD) effluents. AD has been utilised for the stabilisation of municipal solid wastes, but has expanded to the agricultural and agri-food sectors for treatment of animal manures, food waste and horticultural wastes [26–28]. Anaerobic digestion as a technology is recognised as having the following benefits: GHG avoidance from organic waste matter going to landfill, renewable energy production from bio-gas, and recycling of residual organic materials as fertilizers – decreasing reliance on mineral fertilizers [29]. As such, the classification of digestate residuals as a by-product rather than a waste and finding new uses for this resource was seen as a key factor in aiding the development of the AD sector by the UK Department for Environment, Food & Rural Affairs [30]. Thus, the use of ADE as a source of nutrients contributes and exemplifies the principles of a circular economy, which is in line with a number of EU

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initiatives, including 'The Roadmap to a resource efficient Europe' (COM(2011) 574) and the Waste Framework Directive (Directive 2008/98/EC and 2011/753/EU) [31].

Digestates are a sludge that is typically separated into solid and liquid fractions via centrifugation. Solid fractions are enriched in organic nutrients and are composted or used as slow release fertilizer [26,32], whereas the liquid AD effluents (ADE) are a more concentrated source of inorganic N (mainly NH_4) and P-PO_4 [28,33]. As long as the inputs to AD processes are controlled, reactors are operated at high temperature (>55°C, thermophilic operation), and outputs are pasteurised (>75°C for 1 hr) to decrease pathogen loads, these resources can be classified as high-quality fertilizers for use in agriculture for food and feed production [30,34]. Several studies have now demonstrated growth of microalgae on AD waste streams, with results comparable to that of cultures grown on synthetic media [33,35–37]. Coupling of microalgal biomass production to recovery of nutrient rich waste streams from AD is hence an attractive opportunity to mitigate the cost of nutrient inputs, decrease the energy and environmental impact of fertilizer production, while also avoiding competition for potentially limiting resources with other sectors [7,11].

In this study, the use of ADE from the digestion of food waste was assessed as a source of N and P for production of the widely exploited marine microalgae *Nannochloropsis* sp. and the potential effects on biochemical composition, specifically fatty acids. Using the generated empirical data, the cost and environmental savings of utilising ADE nutrients will be considered against a base case of fertilizer inputs.

2   Materials and Methods

2.1   *Anaerobic digestate effluent preparation and composition*

ADE was collected from the commercial scale Biogen Gwyri ~~anaerobic digestion~~AD plant in Gwynedd, North Wales (UK). The plant has an input capacity of 11,000 metric tonnes (t) yr^{-1} of municipal and commercial food waste. Reactors are mesophilic (35–40°C) and generate 3,500 MWh y^{-1} in electrical energy via combined heat and power generators, enough for ca. ~~(approximately)~~ 700

homes yr^{-1}. The sludge removed from the reactor was pasteurised (70°C for 1 hour) by the operators upon removal to decrease pathogen numbers and then the solids and liquid effluent (ADE) were separated using a decanter centrifuge.

A 20 L batch of ADE that had been diluted 50% with deionised water was received in December 2012. The ADE was passed through mesh bag filters to remove solids (Nylon, 1 mm, 100 µm and 10 µm) and then stored at −20°C in 250 mL aliquots to prevent contamination and maintain a constant nutrient composition across all experiments. Aliquots of ADE were defrosted and decanted into 250 mL Erlenmeyer flasks before being sterilised via autoclavation (121°C for 20 mins) for use as culture media. Autoclaved ADE was stored at 4°C and used within 2 weeks.

The nutrient and metal composition of autoclaved ADE is shown in Table 1. Quantification of dissolved nutrients (NO_3, NH_4 and PO_4) was performed using the methods described in section 2.5.2. The pH of the autoclaved ADE was 7.75 at room temperature. The total suspended solids content of the ADE after filtration through a 10 µm filter was measured by filtering 50 mL of the ADE onto pre-combusted (550°C for 20 minutes), pre-weighed 0.7 µm GF/F filters (Whatman, GE Healthcare, Germany). Filters were rinsed with 100 mL of deionised H_2O and then dried at 70°C for at least 18 hrs until a constant weight was recorded. This was performed in triplicate and was determined to be 2.14 ± 0.32 g L^{-1}. Quantification of ADE metal content was performed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after filtering samples through 0.7 µm GF/F filters (Whatman) and dissolution by boiling *aqua regia* digestion. Extraction and analysis was performed by Severn Trent Plc. Analytical Services (Coventry, UK), a UK Accreditation Service approved commercial laboratory.

Table 1. Chemical composition of an anaerobic digestate effluent (ADE) derived from food waste

Macro Elements (mg L^{-1})	
Total nitrogen (TN)	5164
Ammonium	3192
Nitrate	0
Total phosphorus (TP)	136
Phosphate	71
Total Organic Carbon (TOC)	9800
Potassium	150
Calcium	82.2
Magnesium	11
Iron	6.9
Zinc	1.0
Trace Elements (µg L^{-1})	
Arsenic	15.6
Boron	520
Cobalt	15.4
Copper	21
Lead	39
Manganese	73
Molybdenum	16
Sulphur	13
Dissolved sulphides (H$_2$S)	12.7
Tin	18
Titanium	1.8

ADE was filtered through a 10 µm (nylon mesh) prior to analysis of TOC, TN, TP and metals and trace elements. It was also filtered through a 0.7 µm filter (GF/F, Whatman) for analysis of dissolved inorganic nutrients.

2.2   *Algal strain and culture maintenance*

Master cultures of the marine eustigmatophyte microalga *Nannochloropsis* sp. (CCAP 211/78) was maintained in 50 mL Erlenmeyer flasks in a modified seawater based Walne's media [38] containing 20 mg N-NO$_3$ L^{-1} with an N:P ratio of 16:1. P was supplied as NaH$_2$PO$_4$.2H$_2$O. These were sub-cultured approximately every 3 weeks and grown under an irradiance of ca. 50 μmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes (58 W) on an 18:6 hrs light:dark cycle at ca. 18°C. Master cultures were scaled up to 400 mL volume in 1 L Erlenmeyer flasks in preparation for photobioreactor (PBR) trials at 22 ±1°C, with an irradiance of ca. 100 μmol photons m^{-2} s^{-1} with an 18:6 hrs light:dark cycle.

2.3   *PBR system*

The growth system consisted of 10 L tubular airlift PBR (acrylic plastic, 0.1 m diameter/light path, 1.2 m height). Reactors were maintained at 22 ± 1°C, and aerated with filtered ambient air (0.2 μm, 0.039% CO$_2$) at a rate of 0.1 L L^{-1} min^{-1} (v/v) into the base of the tube through a 1 mm inner diameter plastic capillary tube. Bioreactors were illuminated at 100 μmol photons m^{-2} s^{-1} PAR (reactor surface) using daylight fluorescent tubes (T8, 58 W) mounted perpendicular to the bioreactors, and operated under a 18:6 hrs light:dark cycle. Irradiance was measured using a cosine-corrected light meter (WALZ ULM-500).

The seawater base for all media was natural seawater, pumped from Swansea Bay (U.K.), filtered to 1 μm, UV-treated and ozonated. Seawater salinity was 30 ± 2 ppt. The seawater was sterilised in the PBRs via the addition of NaClO (0.5 mL L^{-1}, 15% chlorine) for 8–12 hours before neutralising with Na$_2$S$_s$O$_3$ (0.15 g L^{-1}). Media pH was maintained between 7.8 and 8.0 by addition of 10 mM TRIS-HCl (Melford Chemicals) and 1 M NaOH. Inocula for the growth experiments were grown in the same tubular PBR set-up, which were inoculated from the 1 L Erlenmeyer flask cultures described in the previous section. The inocula were transferred to the experimental cultures to give a starting concentration of 2 x 10^6 cells mL^{-1}. Relative to the final yield, these inocula were ca. 5% of the maximum final cell density, contributing ca. 1.0 mg biomass-N L^{-1} and 0.11 mg biomass-P L^{-1}.

2.4   *Experimental design and media*

This work examined the replacement of different concentrations of N and P in synthetic media (Walne's media) using nutrients contained in ADE. The initial N concentration of treatments supplied with ADE was set at 10 mg N L^{-1}, which was deemed to be the optimum N concentration for the current culture conditions, as it was previously shown to result in rapid growth followed by N-starvation of *Nannochloropsis* sp. within short batch experiments [19]. The media N:P ratio in the first round of experiments was set at 16:1, requiring a P-PO$_4$ concentration of 1.34 mg P L^{-1}. N in the media (initially as nitrate) was replaced at different percentages (25%, 50% and 100%) with N supplied as NH$_4$ as contained in the ADE. The contribution of P-PO$_4$ from ADE at these percentage replacements was then considered (4, 8 and 16% of required P) and additional P-PO$_4$ (NaH$_2$PO$_4\cdot$2H$_2$O) was added to generate media with a 16:1 N:P ratio. A second set of growth experiments were conducted using 100% N replacement by ADE, but with the N:P ratio increased to 32:1 and 64:1. Despite the ADE containing significant quantities of micronutrients (Table 1), Walne's media vitamins and trace metal solutions were also added to prevent possible growth limitation by the potential lack of these components in the ADE. Control treatments consisting of 10 or 60 mg N-NO$_3$ L^1 cultures based on Walnes media (both 16:1 N:P) were also evaluated to compare the effect of ADE nutrients and nutrient starvation on growth and biochemical composition. The 10 and 60 mg N L^{-1} systems are hereby referred to as Cont-N and Cont+N, respectively. Cultures were grown in 10 L column-PBRs for 9 days under the general conditions described in Section 2.3.

2.5   *Analytical techniques*

2.5.1   *Analysis of growth dynamics*

Cell number, total cellular volume and cell size were recorded daily using a Coulter counter (C4 Beckman Coulter GmBH, Drefield, Germany). Culture dry weight (DW) was determined by filtering a known volume of culture onto precombusted GF/F Whatman filters. Filters were washed first with double the volume of ammonium formate (0.5 M) to remove salts, then dried for at least 18 hrs at 70°C, before cooling to room temperature in a desiccator. On a routine basis, DW was estimated

from the Coulter counter-derived biovolume (i.e. total cellular volume) using a previously established calibration of 1 mL cell biovolume (or 1×10^{12} μm^3) = 1 g DW.

Biomass-specific growth rate was calculated from changes in the biomass concentration (mg DW L^{-1}) using the following equation:

$$\mu_{exp} = (\text{In}N_1 - \text{In}N_0) / (t_1 - t_o)$$

where N_0 and N_1 are the biomass concentrations at times t_0 and t_1. The observed exponential (maximum) growth rate (μ_{exp}, d^{-1}) was calculated over the first 4 days of growth (0–4 days).

2.5.2   *Dissolved nutrient analysis*

Nutrient analysis was performed using a Seal Analytical AutoAnalyzer 3 (AA3 HR., Seal Analytical Inc., UK) automated segmented flow analyser interfaced with XY3 Random Access Sampler and controlled using AutoAnalyser Control and Evaluation (AACE) software. Standard methods produced by Seal/Bran Luebbe were used for quantification (NO_3 G-172-96 MT19, NH_4 G-171-96 MT19 and PO_4 G-175-96 MT18). Sample were prepared by filtering 5 mL of culture through A/E filter disks (13 mm, 1 μm pore size; Pall Corporation, NY, USA) and stored at $-20°C$ until analysis.

2.5.3   *Elemental nitrogen and phosphorus content*

At the end of cultivation, a known volume of culture suspension was filtered onto precombusted (550°C for 20 min) A/E glass fibre filter (13 mm, 1 μm). Filters were stored at -80°C and then dried at 60°C for 12–18 hours prior to analysis. Biomass-N content was determined using an elemental analyser interfaced with an isotope ratio mass spectrometer, according to the method described in Mayers et al. [39]; all measurements were made in duplicate and isoleucine was used as a standard. Biomass-P content was measured in cells retained on the filters using the acidic persulfate digestion method (0.015 M $K_2S_2O_6$ plus 0.018 M H_2SO_4, autoclaved at 121°C for 75 min). Biomass-P was converted to free orthophosphate and measured spectrophotometrically using an ammonium molybdate assay at 880 nm with KH_2PO_4 as the standard [40].

2.5.4   Fourier-transform Infrared Spectroscopy (FTIR) biochemical determination

The content of lipids, proteins and carbohydrates in biomass samples collected at the end of the cultivation was determined using FTIR, using the method described in Mayers et al. [39]. Biomass was harvested by centrifugation at 11,000 *g* for 15 mins at 4°C in a Beckman Avanti J-20XP centrifuge with a J-LITE JLA-8.1000 rotor. Biomass was sequentially washed with the same volume as that harvested of ammonium formate and then deionised water to remove salts, and then frozen at -80°C. The frozen biomass was then freeze-dried (ScanVac Cool Safe, LaboGene, Lynge, Denmark) at -110°C for at least 48 hrs, powdered and then freeze-dried for a further 12 hours.

Briefly, freeze-dried microalgal biomass was measured on a PerkinElmer Spectrum Two instrument equipped with a diamond crystal iATR reflectance cell with a DTGS detector scanning over the wavenumber range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}. Three replicates (each of an average of 10 scans) for each sample were taken and the results averaged. Background correction scans of ambient air were made prior to each sample scan. Scans were recorded using the PerkinElmer spectroscopic software Spectrum and quantification methods had been previously built in SpectrumQuant (version 10. PerkinElmer, Germany). Ethanol (60% v/v) was used to clean the diamond ATR between samples.

2.5.5   Fatty acid extraction, transesterification and quantification

The fatty acid (FA) profile and content of biomass samples collected at the end of the cultivation were determined using the direct transesterification method of Laurens et al. [41] consisting of the hydroxyl chlorinated technique to convert FA to fatty acid methyl esters (FAME), which were then separated and measured using gas chromatography equipped with a flame-ionisation detector (GC-FID). Approximately 5 mg of freeze-dried biomass was suspended in 1 mL of 2:1 chloroform/methanol and sonicated on ice for 45 mins. An internal standard containing heptadecanoic acid (C17:0) in methanol was added at a concentration of 250 μg mL^{-1} to each sample, followed by transesterification in 1 mL 0.05% (v/v) hydrochloric acid and 0.1% (w/v) butylated hydroxytoluene (BHT) in methanol at 85°C for 60 mins. The samples were allowed to cool to room temperature and then 2 mL of hexane was added to

extract the FAME for 1 hr. Hexane was removed and an additional 2 mL added then these extracts were combined and dried under nitrogen at 40°C. Samples were then re-dissolved in 2 mL of GC-grade hexane and transferred to GC vials before being analysed by GC-FID (Agilent 6890N, HP-INNOWAX column, 30 m length, 0.32 mm internal diameter, 0.25 μm film; 19091N-113; Agilent, USA). GC run conditions were as follows: temperature program 70 to 300°C at 20°C min^{-1}, plateau for 1 min at 230°C, at a 1.5 mL min^{-1} helium constant gas flow.

Quantification of FAME was based upon integration area of a 5-point calibration curve (0.1–2 μg ml^{-1}) prepared with standards containing 5 even carbon chain FAMEs (FAME Mix GLC-10, 1891-1AMP) and 5 odd carbon FAMEs (FAME Mix GLC-90, 1896-1AMP). Individual FAMEs were identified based upon comparisons of the retention time of FAME standard mix (Supelco 37 Component FAME mix; Sigma, CRM47885). Concentrations were normalised against the internal standard of 250 μg mL^{-1} heptadecanoic acid (C17:0).

2.6   *Life cycle assessment (LCA) and cost savings*

This analysis considered the cumulative energy demand (CED), global warming potential (GWP) as CO_2-equivalents and the monetary cost of fertilizer inputs, and how they can be mitigated by the use of nutrients contained in the ADE used in this study. The cost of different N and P based fertilizers was taken from the Average U.S. farm prices of selected fertilizers for 2013 [18]. The GHG footprint as CO_2-equivalents and cumulative energy demand of fertilizers were taken from inventory of EcoInvent Version 3.0 database (Table 2) [42].

The system boundaries for this analysis were limited to the supply of N and P nutrients for algal biomass production; it does not include the energy costs of other nutrients, cultivation or downstream processing. However, the loss of biomass and lipid during biomass processing are accounted for in the calculation of final nutrient requirements. There is considered to a loss of 5% of biomass during harvesting [8]. The conversion of lipids in biomass to biodiesel was assumed to be 90% based on the recent progress made in direct transesterification of wet biomass of *Nannochloropsis* species [43]. The cost and energy demand of transport for all materials was negated,

as was the potential requirement for treatment of ADE nutrients, such as filtration or centrifugation. The functional units were per tonne of dry biomass (t DW) and per tonne of biodiesel (t fuel).

Three different nutrient requirement scenarios were considered: Replete, Deplete and Redfield, as outlined in Table 5. The replete scenario uses the N, P and lipid data generated by the Cont+N treatment, the deplete scenario represents nutrient starved biomass grown at an increased N:P molar ratio of 32:1 that was previously determined to be suitable for growth of *Nannochloropsis* sp. by Mayer et al. [19]. To aid comparison with other studies, the nutrient requirements predicted by the popular Redfield ratio, as used in a number of LCA was also considered (C_{106}:N_{16}:P_1 assuming a biomass C content of 50% DW; Table 5) [8,44]. It is presumed biomass with such an elemental composition is not N or P-stressed and would subsequently have a low lipid content, as per the replete scenario. For each scenario, the base case assumes all nutrient demands are met by fertilizer nutrients, whereas the alternate strategy assumes that ADE meets N requirement and a corresponding quantity of P, with the remaining P supplied by monoammonium phosphate, with the N-NH_4 in MAP (0.11 kg N kg^{-1} fertilizer) also considered. ADE supplied over 94% of N in all scenarios (Table 5).

The fertilizers considered were ammonium nitrate (NH_4NO_3) and MAP ($NH_4H_2PO_4$). The contribution of NH_4 from MAP for the fertilizer cases was also considered, meeting 5–15% of total N for the different scenarios. It was found that meeting N and P in this way had a lower cost, CED and GHG than not accounting for the N in MAP, or using a P source containing no N, such as triple super phosphate, which has a higher impact than MAP. The CED, GWP and cost of AN and MAP are displayed in Table 2.

Table 2. Fertilizer input impacts in per tonne of N or P of material

	CED (MJ)	GWP (CO_2-eq)	Cost ($)
Ammonium nitrate	67.8	9.37	1.71
Monoammonium phosphate	31.2	1.49	3.39

CED = cumulative energy demand, GWP = global warming potential. CED and GWP were taken from the EcoInvent LC~a~A inventory database, while costs were representative of 2013 U.S. prices.

2.7 Statistical analysis

Differences in treatments were assessed by one-way analysis of variance (ANOVA). If ANOVA results were significant ($p < 0.01$), comparisons between means were made using Tukey's post hoc test. Statistical analyses were conducted using the software R (version 0.97.551). The number of statistical replicates was at least three for all analysies, unless otherwise stated in the figure legend for specific data sets.

3 Results and discussion

3.1 Growth dynamics of ADE supplied cultures

There were no significant effects on the exponential growth rate (day 0 to 4) and biomass productivity (over 4 days) resulting from the use of ADE at any concentration tested, compared to control media (Table 3; Fig. 1). Exponential growth was found to begin within the first day of cultivation, with maximum growth rates between 0.45–0.56 d^{-1} (corresponding to a generation time of 1.25–1.55 d). This indicates that no components of the ADE were inhibitory at the concentrations supplied, nor were any significant negative effects found due to media turbidity and light absorption caused by suspended solids (>10 µm). High media turbidity when using of waste waters has previously been found to have an inverse relationship with algal growth rates [35]. Cai et al. [36] found that *Nannochloropsis salina* had a significantly lower growth rate at high loading levels of a municipal waste ADE (18–24% v/v; 0.33 d^{-1}) when compared with lower loading rates (3% v/v, 0.62 d^{-1}) when grown at an irradiance of 200 µmol m^{-2} s^{-1}. The growth rates reported here (0.53 d^{-1} at 0.08–0.31% v/v; Table 3) are slightly lower than those of the highlighted study of Cai et al. [36], but this is expected when the difference in irradiance is considered. These results are however similar to the growth rates of *Nannochloropsis* sp. when grown on municipal wastewater (0.54 d^{-1}) at similar irradiances to that used in this study [45]. Overall these growth rates and biomass productivities fall quite short of those achieved in other studies using *Nannochloropsis* species when cultures have been supplied with additional CO_2 and higher irradiances. For example, under a similar irradiance to this study (~100 µmol photons m^{-2} s^{-1}), but with additional CO_2 supplied, Van Wagenen et al. [46] achieved biomass productivities of over 100 mg L^{-1} d^{-1} for *N. salina* (~50 mg L^{-1} d^{-1} in this study).

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Furthermore, productivities of over 500 mg L^{-1} d^{-1} have been achieved in large scale outdoor cultivations of *Nannochloropsis gaditana* in in Southern Spain [47]. This suggests greater productivities are certainly obtainable through optimisation of CO_2 supply and irradiance.

Anaerobically digested food waste was also recently assessed as a nutrient source for growth of *Scenedesmus bujuga* [48], achieving biomass productivities was equal to that on synthetic media (46–51 mg L^{-1} d^{-1}; BG-11 media) at an optimum dilution of the waste stream (5% v/v). However, at the highest concentration of effluent (10% v/v equalling 120 mg N-NH_4 L^{-1}), biomass productivity decreased by 14–20% versus the most productive treatments. This could results from either high media turbidity or high concentrations of ammonium that may have inhibited growth. Although there appears to have been no negative effects due to turbidity in the current study, it is possible that at higher ADE concentrations (or different ADE batches) this may become an issue in achieving high initial growth rates.

All treatments supplied with 10 mg N L^{-1} became nutrient limited by day 6 (Fig. 1C), coinciding with a cessation of cell division (Fig. 1A). These treatments all had the same yield of biomass on N supplied (g DW g N^{-1}; Table 3). However, in the Cont+N treatment, which was supplied with 60 mg N L^{-1}, approximately 50% remained on day 9, resulting in a significantly lower yield of biomass on N compared to the other treatments. This suggests the continued accumulation of biomass in the form of lipids and carbohydrates following N depletion in low nutrient treatments, as has been seen in many other studies [49–51]. The biomass yield on P was also different between treatments, with Cont+N having a significantly lower yield than the other treatments, with a final P content of 1.2% DW compared to 0.3–0.4% DW for the low N supplied treatments.
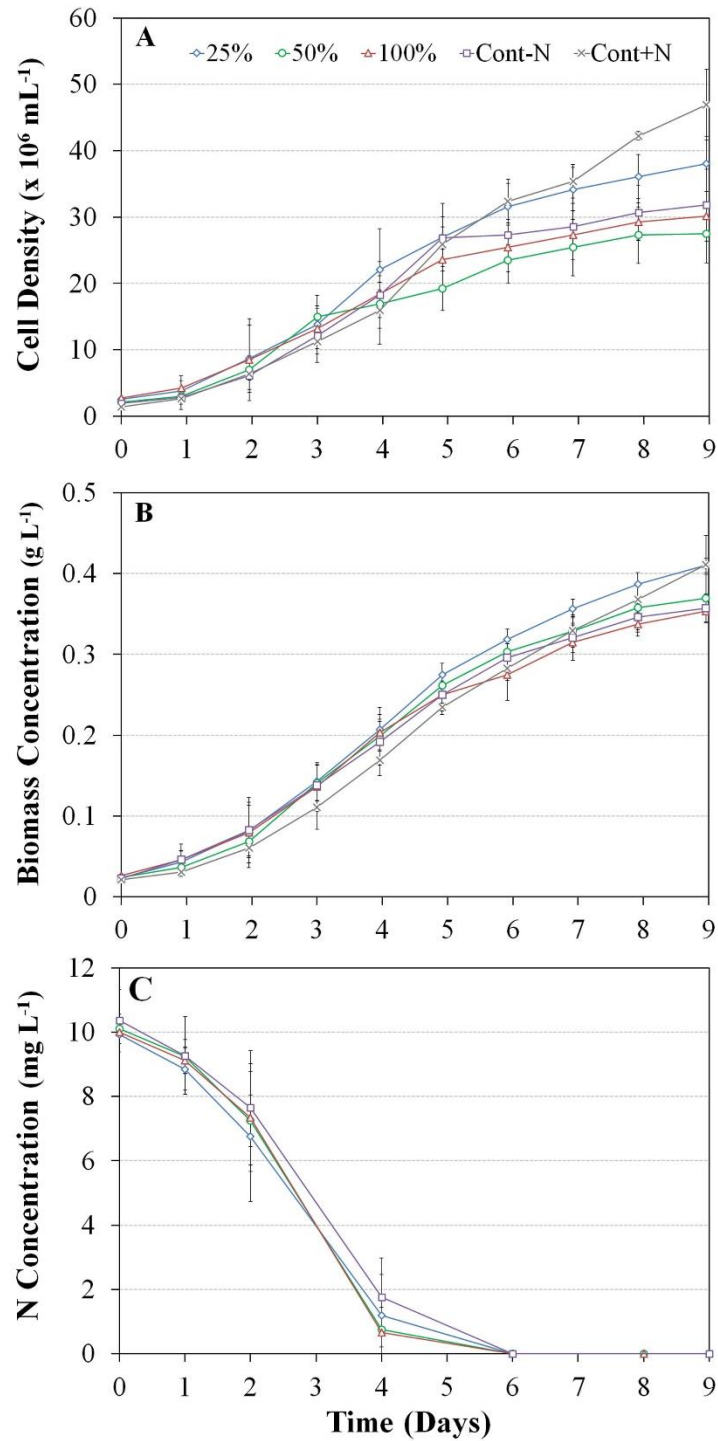
Figure 1. Growth curves of *Nannochloropsis* sp. cell number (**A**; x 10^6 mL^{-1}) and dry biomass concentration (**B**; g DW L^{-1}), and the residual media N concentration (**C**; mg L^{-1}) for batch cultures grown on different percentages of media nitrate (25, 50 and 100%). Residual N concentration is the sum of both NO_3 and NH_4. Cont-N and Cont+N treatments both had NO_3 as the nitrogen source (ADE treatments and Cont-N = 10 mg N L^{-1}; Cont+N = 60 mg N L^{-1}). Residual N data for the Cont+N treatment not shown, but was > 30 mg N L^{-1} on day 9 (n ≥ 3, mean ± 1 SD).

Table 3. Growth parameters of *Nannochloropsis* sp. batch cultures grown with different percentages of N replaced by ADE. Cont-N and Cont+N treatments both had NO_3 as the nitrogen source (ADE treatments and Cont-N = 10 mg N L^{-1}; Cont+N = 60 mg N L^{-1}; .n ≥ 3, mean ± 1 SD).

Parameter	25% ADE	50% ADE	100% ADE	Cont-N	Cont+N
Exponential growth rate (d^{-1}) *	0.52 ± 0.06	0.53 ± 0.04	0.51 ± 0.03	0.53 ± 0.05	0.55 ± 0.03
Maximum biomass concentration (g DW L^{-1})	0.414 ± 0.007 [a]	0.370 ± 0.030 [ab]	0.353 ± 0.014 [ab]	0.357 ± 0.017 [b]	0.411 ± 0.036 [ab]
Maximum biomass productivity (g L^{-1} d^{-1}) *	0.058 ± 0.003	0.056 ± 0.001	0.052 ± 0.003	0.052 ± 0.004	0.051 ± 0.001
Biomass N:P ratio	17.0 ± 2.1 [a]	18.4 ± 2.2 [a]	15.8 ± 0.5 [a]	19.1 ± 2.2 [a]	9.2 ± 0.8 [b]
Biomass per unit N (g DW (g N)$^{-1}$)	39.2 ± 1.4 [a]	33.6 ± 3.3 [a]	36.4 ± 2.6 [a]	29.7 ± 2.8 [a]	19.2 ± 0.7 [b]
Biomass per unit P (g DW (g P)$^{-1}$)	300.3 ± 30.0 [a]	277.5 ± 13.9 [ab]	261.8 ± 25.9 [ab]	250.5 ± 11.3 [b]	79.4 ± 4.4 [c]

Different letters indicate statistically significant differences between treatments ($p < 0.01$) as tested using one-way ANOVA and Tukey's Post-hoc test. * indicates no statistical difference between treatments.

These experiments also demonstrate that this species is able to assimilate NO_3 and NH_4 equally well, and could flexibly switch between the two with no demonstrable change in growth dynamics, as was the case in the 25 and 50% ADE treatments. There are differing results in the literature regarding the N-source resulting in the highest productivities for *Nannochloropsis* species. Some studies have shown either higher growth rates on NO_3 [52] or NH_4 [53,54], or as is the case in this study no detectable difference [55,56]. It is likely that species or strain specific differences, or the different culture conditions applied in these experiments (*e.g.* irradiance or culture pH) may be responsible for these different observations. Additionally, it was found that pre-acclimation to a specific N-source had a significant improvement on growth rates on that N source [54], a factor that is rarely considered in many studies. The flexibility of this strain with regards to metabolism of different N sources is a useful trait if complex wastewaters with varying mixtures of N-sources are utilised.

Experiments using in which ADE was supplied at 100% but with higher N:P ratios (32:1 and 64:1) were also conducted to determine if nutrient usage could be further decreased. Growth rates at

the higher ratios were comparable to at the 16:1 N:P ratio (0.51–0.58 d^{-1}; Supplementary Materials Table 1). The 32:1 and 64:1 treatments had significantly lower biomass P contents than the 16:1 treatments (0.16 versus 0.3–0.4% DW, respectively; $p < 0.01$; Supplementary Materials Table 1), but all treatments had approximately the same N content (~3% N DW; $p > 0.01$). This highlights that P is supplied in abundance if N and P fertilizers are supplied at the Redfield ratio- (or those of other common laboratory media, e.g. 25:1 in Guillard's F/2 media) as has previously been demonstrated [20,51,57].

ADE nutrient concentrations are found to vary depending on inputs and process configuration [28]; subsequently, close monitoring of ADE nutrient concentrations at the point of production (*i.e.* AD plant) or following storage, would be recommended. In this way,s ADE dilution can be optimised and the requirements for supplementation with additional nutrients (*i.e.* P or trace metals) can be determined to yield predictable growth rates and biomass yields. This also highlights a limitation of the current study, in that we have only investigated a single ADE sample and which does not account for seasonal variations in composition and the effect on biomass production. The high N:P ratio of the ADE (99:1) used in this study was not ideal for biomass production and required addition of significant quantities of inorganic P to provide an appropriate N:P. Conversely, if the concentration of NH_4 in the ADE is high and supplied in high levels to microalgal cultures, this may become inhibitive to growth depending upon the strain [56]. *Scenedesmus* sp. was found to tolerate relatively high concentrations of up to 100 mg N-NH_4 L^{-1} [58], but concentrations significantly below this level were found to be inhibitory to *Neochloris oleoabundans* and *Dunaliella tertiolecta* [56]. Identifying AD processes that produce effluents with relatively consistent nutrient outputs with a low N:P ratio should be a priority in selecting the nutrient streams most appropriate for utilisation.

3.2 Biochemical composition

The biochemical composition of nutrient replete *Nannochloropsis* sp. biomass was typical of non-nutrient starved cultures, having a high protein content and a low lipid/carbohydrate content (Fig. 2A) [19]. In contrast the biochemical composition of treatments with low N supply (10 mg N L^{-1}) indicates that they were severely N-starved, with high lipid contents (>42% DW) and low protein contents (<21% DW). The ADE treatments had considerably higher lipid and carbohydrates contents than the Cont-N treatments ($p < 0.01$), but only the 25% ADE had a correspondingly lower protein content. *Nannochloropsis* species are known to be oleaginous and the high lipid contents reported here are not uncommon under N-starvation [59–61]. The accumulation of lipids and carbohydrates following N-depletion is brought about by a cessation of cell division, as *de novo* protein synthesis is halted. C, energy, and reductant is then redirected to the synthesis of macromolecules containing no N. Lipids and carbohydrates are also more dense electron sinks compared to proteins [62], providing a sink for both the excess energy absorbed by the photosystems and subsequent excess reducing potential [63–65]. This represents another mechanism that aids in the dissipation of excess energy, cellular redox rebalancing, and preventing radical oxygen species formation [65–67].

In terms of volumetric concentrations of biochemical groups, the 25% ADE treatments produced significantly greater quantities of lipids (228 mg L^{-1}) and carbohydrates (80 mg L^{-1}) than the other N-limited treatments, which corresponded to a greater volumetric productivity of lipids (25 mg L^{-1} d^{-1} versus 18-20 mg L^{-1} d^{-1}, Fig. 2B). The higher volumetric concentrations for lipid and carbohydrate in the ADE grown cultures compared to the control are likely due to the combined effect of small differences in biochemical composition and more significant differences in the final biomass concentration (Fig. 2B). The Cont+N treatment had a significantly greater protein productivity than nutrient limited treatments ($p < 0.01$; Fig. 2B) owing to continued protein synthesis in the non N limiting media. The lipid content of 100% ADE treatments grown at higher N:P ratios was approximately the same as those grown at 16:1 (50–52% DW; $p > 0.01$; Supplementary Materials Table 1) in accordance with previous findings [51].
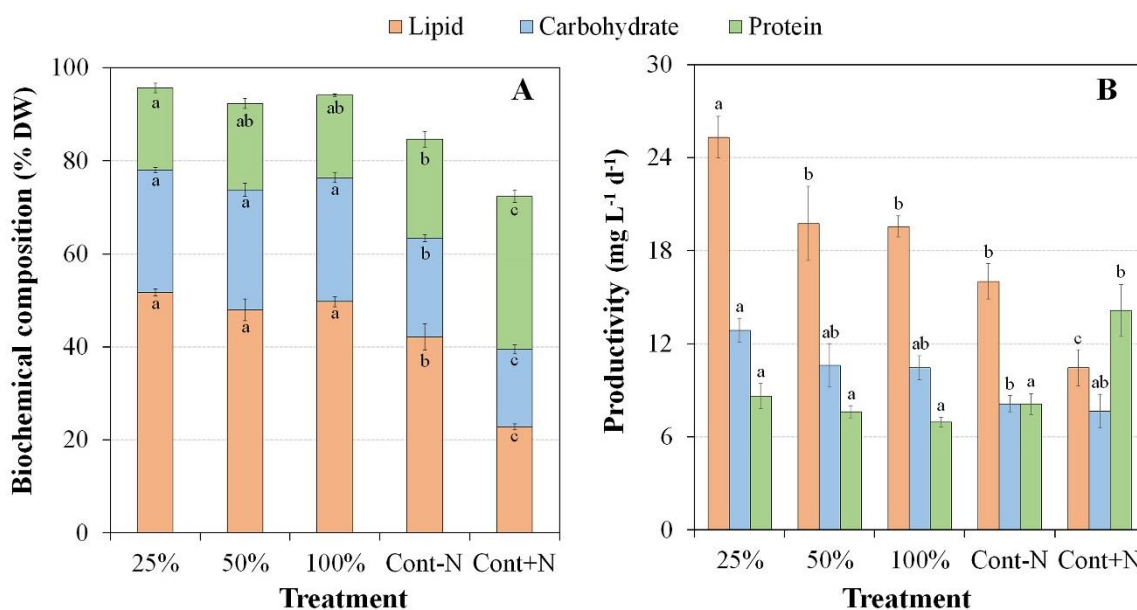
Figure 2. *Nannochloropsis* sp. biomass biochemical composition (A;% DW; lipids, proteins and carbohydrates) and the productivity of these components (B; mg L^{-1} d^{-1}) on the last day of batch cultivation with difference percentages of total N replaced by ADE. Cont-N and Cont+N treatments both had media with NO_3 as the nitrogen source (ADE treatments and Cont-N = 10 mg N L^{-1}; Cont+N = 60 mg N L^{-1}, n ≥ 3, mean ± 1 SD). Different letters represent statistically significantly differences between treatments for a given biochemical component ($p < 0.01$; One-way ANOVA and Tukey's post hoc test).

3.3 Fatty acid profile

The FA profile of N-replete biomass shows C16:0, C16:1 and C20:5 to be the dominant FA, consistent with that of other *Nannochloropsis* species [60,61]. In N-limited treatments, these FA were all still very highly represented, but the percentage of C18:1 also increased (Table 4). The fatty acids that showed the greatest increase as a percentage of dry weight under N-limitation were C16:0 and C18:1 (ca. 3-fold and 5-fold, respectively). There were small variations in the FA profile between ADE grown treatments, but overall there were no significant differences in the TFA content (Table 4; $p > 0.01$). The FA content and profile of *Nannochloropsis* sp. subsequently appears to be largely unaffected by the nutrient source or other compounds contained in the ADE. This was also found in the study of Sheets et al. [68], which compared biomass production and the FA profile of *N. salina*

grown on media composed of anaerobically digested municipal waste or synthetic media supplied at the same nutrient concentration (200 mg N L^{-1}).

The content of C20:5 or eicosapentaenoic acid (EPA), a commercially important omega-3 fatty acid, was the same in all treatments (7.2–8.7% DW), with a peak productivity of 1.7–2.8 mg EPA L^{-1} d^{-1}. These values compare favourably to those in the literature for batch cultivations given the cultivation system employed in this study [69,70], but fall short of the productivities of *Nannochloropsis* species grown in continuous cultures with higher irradiances and increased CO_2 supply, which can be greater than 10 mg L^{-1} d^{-1} [47,71].

The only significant difference seen between the ADE treatments was that cultures grown on 50% ADE had a greater quantity of SFA than the other treatments, mainly comprised of increases in the quantities of C14:0 and C16:0. The recent study of Racharaks et al. [72] found that *N. salina* grown on mixed N-source (NH_4 and NO_3; modified F/2 media) had a greater proportion of SFA than cultures grown on just NH_4 (but with the same N concentration), which was supplied by digested municipal waste. It might be that the effect of growth on an equal mix of the two N-sources as done here could be responsible for the difference in FA profile seen in the 50% ADE treatments, but elucidating the physiological reason is not possible with the current experimental data. Further attention should subsequently be given to the effect of N-sources (NO_3 versus NH_4) on *Nannochloropsis* fatty acid metabolism.

The fatty acid profile of *Nannochloropsis* species has previously been determined to be satisfactory for biodiesel production, but may suffer from poor oxidative stability due to high concentrations of poly-unsaturated fatty acids (PUFAs) [73,74], so it is preferable to utilise nutrient starved biomass to decrease the proportion of these components in the final oil.

Table 4. Fatty acid (FA) composition as a percentage of dry weight of *Nannochloropsis* sp. lipids grown in batch cultures with different concentrations of ADE and harvested at stationary phase. Controls cultures (Cont-N and Cont+N) were grown on NO_3 (Cont-N and ADE = 10 mg N L^{-1}; Cont+N = 60 mg N L^{-1}; n ≥ 3, mean ± SD)

FA (% of DW)	25% ADE	50% ADE	100% ADE	Cont-N	Cont+N
Total fatty acid (TFA)	40.5 ± 4.8 [a]	41.0 ± 3.8 [a]	36.2 ± 2.2 [a]	36.0 ± 2.7 [a]	21.3 ± 2.5 [b]
Myristic acid (C14:0)	1.6 ± 0.1 [ab]	2.4 ± 0.5 [a]	1.6 ± 0.2 [ab]	1.2 ± 0.3 [b]	0.9 ± 0.0 [a]
Palmitic acid (C16:0)	14.6 ± 1.3 [ab]	17.1 ± 1.1 [a]	13.9 ± 1.1 [b]	13.5 ± 1.2 [b]	4.7 ± 0.4 [c]
Total Saturated (SFA)	16.6 ± 1.4 [a]	19.8 ± 1.5 [b]	15.9 ± 1.4 [ab]	15.0 ± 1.3 [a]	5.6 ± 0.4 [c]
Hexadecenoic acid (C16:1$_{\Delta 9}$) *	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	0.9 ± 0.5
Palmitoleic acid (C16:1$_{\Delta 7}$)	9.9 ± 0.2 [a]	11.0 ± 0.9 [a]	9.7 ± 0.5 [a]	9.7 ± 0.8 [a]	5.4 ± 0.3 [b]
Oleic acid (C18:1)	2.8 ± 0.5 [a]	2.4 ± 0.7 [a]	2.9 ± 0.3 [a]	3.0 ± 0.3 [a]	0.7 ± 0.1 [b]
Linoleic acid (C18:2)	0.8 ± 0.1 [ab]	1.0 ± 0.1 [ab]	0.8 ± 0.1 [ac]	0.6 ± 0.1 [b]	0.4 ± 0.0 [b]
Linolenic acid (C18:3) *	0.1 ± 0.0	< 0.1	< 0.1	< 0.1	< 0.1
Arachidonic acid (C20:4, ARA) *	1.1 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.0
Eicosapentaenoic acid (C20:5, EPA) *	5.5 ± 1.0	5.1 ± 1.3	5.3 ± 1.4	6.2 ± 0.8	7.3 ± 1.2
Total Unsaturated (UFA) *	20.9 ± 1.0	21.2 ± 2.7	20.5 ± 2.5	21.2 ± 1.7	15.6 ± 2.1
Total Monounsaturated (MUFA)	13.4 ± 0.4 [a]	14.0 ± 1.3 [a]	13.2 ± 0.9 [a]	13.3 ± 1.0 [a]	7.0 ± 0.8 [b]
Total Polyunsaturated (PUFA) *	7.4 ± 1.2	7.2 ± 1.7	7.2 ± 1.6	7.8 ± 0.7	8.7 ± 1.3
TFA productivity (mg TFA L^{-1} d^{-1})	17.9 ± 2.7 [a]	16.5 ± 1.8 [a]	13.0 ± 1.7 [a]	15.5 ± 1.1 [a]	9.8 ± 2.0 [b]
EPA productivity (mg EPA L^{-1} d^{-1}) *	2.6 ± 0.7	2.1 ± 0.5	1.7 ± 0.6	2.7 ± 0.3	2.8 ± 0.5

Different letter indicate statistically significant differences between treatments ($p < 0.01$) as tested using One-way ANOVA and Tukey's post-hoc test. * indicates no statistical difference between treatments.

3.4 Cost, energy and GHG savings from ADE usage

The nutrient requirements predicted by the biomass content of N and P from these experiments are used as the basis to calculate the potential savings of energy, CO_2 emissions and financial costs associated with fertilizer use. For the fertilizer case, AN and MAP were used as N and P sources (Table 2), with the contribution of NH_4 from MAP accounting for between 5–15% of total N requirements for the different nutrient scenarios (Supplementary Materials Table 2). The nutrient requirements suggested by the Redfield ratio ($C_{106}:N_{16}:P_1$) when a 50% C content in biomass is assumed [75], are significantly greater than those predicted in the experiments performed here for *Nannochloropsis*. In many techno-economic assessments and LCA of microalgae, the use of the Redfield ratio has led to overestimations of the nutrient requirements of large scale production [8,11]. On the other extreme, a recent review found that 25% of LCAs lack any nutrient requirements in the inventories at all [76]. Here we included the nutrient concentrations predicted using the Redfield ratio to illustrate how exaggerated nutrient requirement data can negatively skew cost, energy and GWP footprints. A nutrient replete biomass production scenario (not being N-starved and having a low lipid content; Table 5), and one in which the culture is N-limited and with an increased N:P ratio (having a higher lipid content) were also assessed to highlight the effect of biomass compositions in process assessments.

Table 5. Compositional data for the different scenarios considered for the impact of nutrient supply

Nutrient Scenario	N / P content (% DW)	Lipid Content (% DW)	Total N input (kg t DW^{-1})	Total P Input (kg t DW^{-1})	Volume AD per t DW (m^3)	Percentage of nutrients met by AD (%)	
						N	P
Redfield[a]	8.8 / 1.2	20[b]	92.4	12.6	27.3	94.4	15.4
Replete	6 / 0.4	20	63	4.2	19.3	97.8	32.6
Deplete	3 / 0.16	50	31.5	1.68	9.72	98.5	41.1

[a] assumes 50% carbon content. [b] predicted lipid content. Nutrient inputs calculated by accounting for 5% loss of biomass during harvesting.

As expected, due to the greater nutrient requirements, the Redfield nutrient scenario has a higher cost, energy demand and GHG-footprint compared to the replete and deplete scenarios for both biomass and biodiesel (Fig. 3). The use of the Redfield ratio results in >140% greater estimation of cost, energy demand and GWP compared to the replete scenario for biomass production and even greater overestimations for biodiesel, even though both scenarios have the same lipid content. The deplete scenario decreases the impact of these categories by at approximately 50% compared to the nutrient replete scenario for biomass, and by 80% for biodiesel. The nutrient-deplete scenario performs proportionally better for the biodiesel production case than for biomass in comparison to the replete scenario owing to the higher lipid content. Biomass lipid content has been determined as a key factor affecting cost and sustainability criteria in a number of studies [10,77,78]. A "pond-to-pump" TEA by Rogers et al. [78] found that an increase in lipid content from 25% to 35% decreased the total cost of biodiesel production by 29%.

Replacement of fertilizer N with that in contained ADE results in 15, 33 and 41% of P replacement replaced for the Redfield, replete and deplete scenarios, respectively. The remaining P requirement is subsequently made up by MAP, with the contribution of N from this fertilizer also considered and the new N-requirement recalculated. The final percent replacements of N by ADE for the different nutrient scenarios is greater than 94% (Table 5). The percentage decrease in the considered impact factors when using ADE versus fertilizer is presented in Fig. 3. The savings across all impact factors for N and P replacements is considerable for the replete and deplete biomass scenarios, with costs decreased by 93–95% (Fig. 3 A1 & A2), and at least 99% decrease in energy usage (Fig. 3 B1 & B2), and GWP footprint (Fig. 3 C1 & C2).
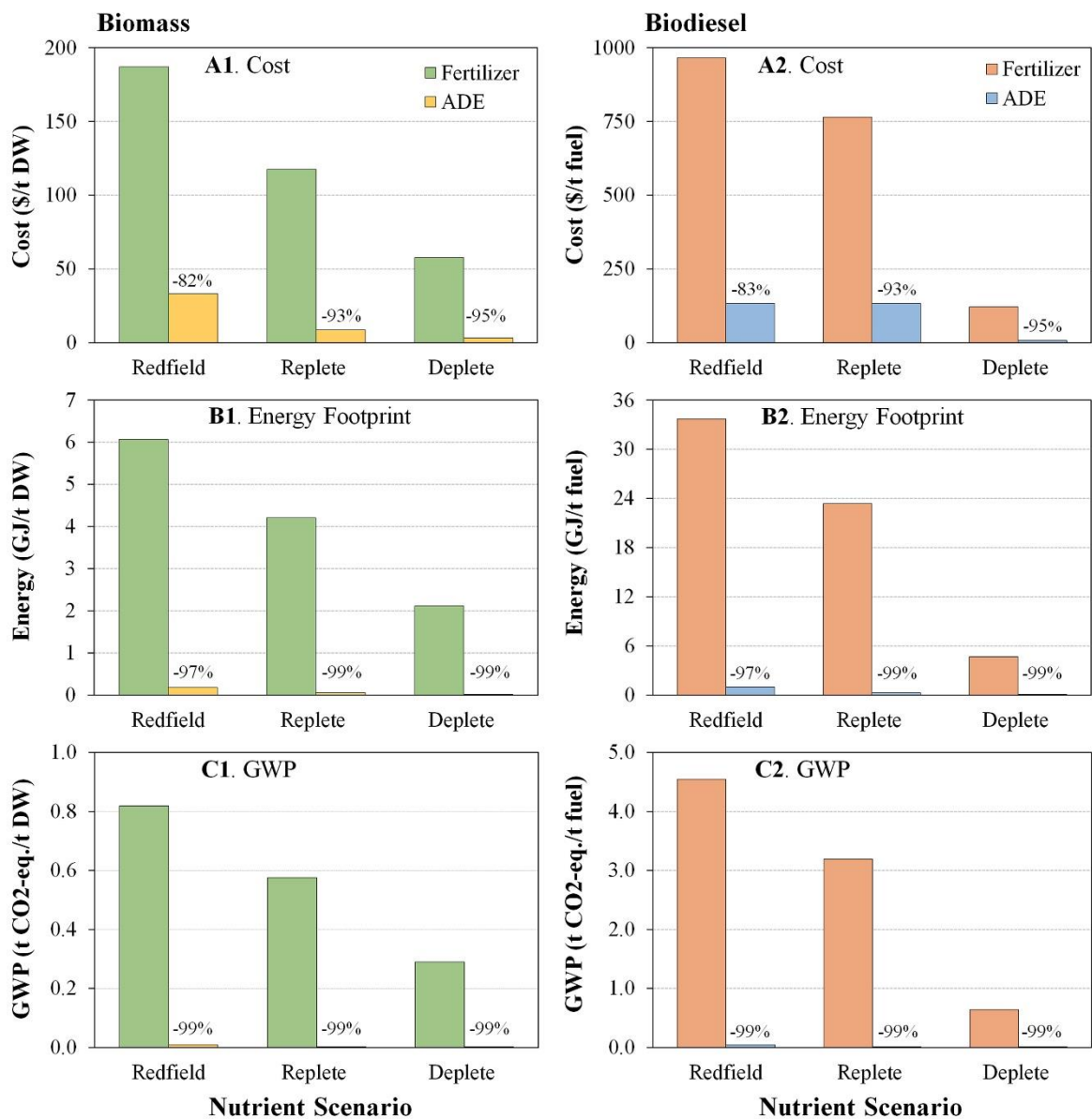
Figure 3. Impact of fertilizer usages and nutrient replacement using ADE on cost (A – top row), energy demand (B – middle row) and global warming potential (C – bottom row) for biomass production (A1, B1, C1 – left column) and biodiesel production (A2, B2, C2 – right column) production for the Redfield, Replete and Deplete nutrient scenarios. Nutrient scenarios are based on biomass composition data shown in Table 5.

The contributions of N and P in the three scenarios were considered as percentages of the inherent energy of biomass and biodiesel to aid in placing the impacts of nutrient inputs in perspective, but it is obviously worth bearing in mind that recycling of N and P within a microalgal biorefinery for biofuel production is essential [6]. The energy content of microalgal biomass is assumed to be 24 GJ t DW^{-1}, and the energy demand for fertilizer nutrients can subsequently account for as much as 25% of this for the Redfield scenario, but only 9% for the deplete scenario. For biodiesel (40 GJ t $fuel^{-1}$), nutrients make a considerable contribution, with even the deplete fertilizer scenario accounting for 12% of biodiesel energy. These energy demands could significantly impact the energy return on investment of bioenergy production from microalgae. When considering the overall energy of biodiesel production (including cultivation and processing), nutrients have previously been found to account for as much as 14–61% of the total energy demand [5,77,79–81]. In this case, the use of ADE decreases nutrient input energy demand to less than 0.8% and 2.5% of the energy content of the biomass and biodiesel, respectively. Handler et al [11] calculated that the contribution of fertilizers to the GHG emissions of biomass production for nine LCA covering over a dozen production scenarios, was between 8.2–75% of total emissions. This large spread of values highlights the challenges faced by LCA practitioners in predicting accurate energy requirements and GWP for biomass production for technology platforms still in the relatively early stages of development.

In terms of cost, the price of a tonne of biomass is hard to value without an application specified and was subsequently omitted from this analysis. For the cost of producing algal biomass (including the use of fertilizers), recent TEA predict values of between $670–12900 per t DW, owing significant differences in areal biomass productivities, system designs, biomass harvesting techniques and labour requirements [82–84]. Tredici et al predicted that the price of fertilizers per t DW was >$230, which is higher than that predicted here as they modelled the use of most expensive N and P sources ($NaNO_3$ and NaH_2PO_4) [83]. This represented only 2% of the cost of operating costs (including yearly depreciation) of biomass production for a 1 ha algal production facility using flat panel PBRs located in Northern Italy. Hoffman et al. state fertilizer costs of just $104 t DW^{-1}, lower than that predicted here despite having comparable nutrient requirements, but still representing 15.5%

of the total cost of production for open ponds situated in the South Eastern US. For biodiesel, which fluctuated between \$900–1000 fuel t^{-1} in 2016 [85], the nutrient costs represent nearly the entire value of the fuel for the Redfield scenario (~\$950 t fuel^{-1}; Fig. 3 A2), 69% for the replete scenario, and 14% for high lipid/nutrient deplete biomass. ADE use would decrease these costs to 0.7 and 4.9% of the biodiesel value for deplete and replete scenarios, respectively. Recovering nutrients from waste streams has been estimated to decrease the cost of production of biomass production by 1–16% [86] and biodiesel by as much as 5–32% [77,87], with variation accounting for different growth systems.

The transport of ADE nutrients has not been considered in this study, as we believe that co-location with an AD operator of an appropriate size would be feasible. It is expected that if transportation were required, it would add significantly to the cost and environmental impact of using ADE nutrient resources due to their relatively dilute nature compared to fertilizers [26].

Although it has been demonstrated that the use of residual nutrient sources makes significant savings compared to the use of fertilizers, this may not necessarily translate to significant decreases in cost, GHG footprint or improving the energy return on investment for the entire production process. This is demonstrated in the large variability in percent reductions highlighted in the literature. Nevertheless, sourcing of fertilizers for large scale algal biomass production to meet our energy or food requirements will certainly have significant impacts on international fertilizer supply and production. As already described, replacing 25% of US transport fuels with algal derived fuels would consume up to 25% of US fertilizer production [6], a similar picture has emerged in the EU [88], highlighting the importance of identifying and utilising sustainable nutrient sources. However, recent analyses in the US has shown that wastewater resources alone cannot meet the nutrient requirements to fulfil the same targets [6,10]. Subsequently a range of solutions are required to meet nutrient demands of large scale production. These include improved nutrient recycling within algal biorefineries possibly by either anaerobic digestion of the residual biomass fraction post-lipid extraction or thermochemical processing of biomass to valorise nutrients in the biomass [6,10]. Although these strategies may negatively influence profitability by resulting in the loss of a potentially higher value co-product, such as animal feed [5,79]. Ultimately, for truly massive microalgal biofuels

production, near-100% recycling of N and P will be required from the biomass that is not recovered for fuel production. This will most likely involve either a biological (ADE-like) approach, or a chemical engineering intervention.

Through our own analysis and those of other studies, it is apparent that nutrient supply for biomass production is a significant contributor to energy demand, GHG emission and the cost of production. The use of ADE and other sources of low impact nutrients from wastes represents a key factor in decreasing the costs and environmental impact of biomass production, while decreasing reliance on potentially sensitive resources. This is a demonstration of increased circularity in the bioeconomy, ensuring materials or nutrients aren't wasted, while improving the sustainability of other production chains. Such activities are essential in the development of a sustainable future bioeconomy.

4.   Conclusion

In this study, we have demonstrated the successful utilisation of nitrogen and phosphorus derived from the effluents of anaerobically digested food waste for the production of *Nannochloropsis* sp. biomass. Growth and biochemical compositions, including lipid content and fatty acid profile, were comparable to that of cultures produced using defined laboratory media. The N:P ratio of media including ADE could also be increased to 32:1 without significant impact, further decreasing P usage. The cost, energy demand and greenhouse gas emissions of fertilizer use was calculated and compared to a scenario utilising ADE nutrients for different compositions of biomass. The use of the Redfield ratio overestimates >140% the contributions of nutrient use across all impact factors compared to the nutrient requirements determined from empirical data in this study. The use of empirical data generated in this study regarding the use of ADE as a nutrient source showed that the impact of nutrient requirements associated with fertilizers could be decreased by at least 90% for cost, energy input and GHG emissions. It is likely that the use of residual nutrients in ADE is currently only viable for biofuel production given current legislation. Surveying of ADE nutrient outputs will suggest the potential of this resource for large-scale cultivation, but factors such as suitability for biomass production need to be considered with regards to the end use of the biomass. Utilising nutrients from

AD sources has potential to increase linkage in bio-based processes as part of a future circular bio-economy.

References

[1]   J. Ruiz, G. Olivieri, J. de Vree, R. Bosma, P. Willems, J.H. Reith, M.H.M. Eppink, D.M.M. Kleinegris, R.H. Wijffels, M.J. Barbosa, Towards industrial products from microalgae, Energy Environ. Sci. 9 (2016) 3036–3043. doi:10.1039/C6EE01493C.

[2]   S. Leu, S. Boussiba, Advances in the Production of High-Value Products by Microalgae, Ind. Biotechnol. 10 (2014) 169–183. doi:10.1089/ind.2013.0039.

[3]   J.C. Quinn, J. Hoffman, R. Pate, T. Drennen, B. Fogleman, R. Davis, A. George, B. Wu, P. Pacheco, M. Zivojnovich, Economic Feasibility and Life Cycle Impact of a Turf- Scrubber Based Biorefinery, in: Algal Biomass Summit (Conference), Washington, DC (September-October, 29 - 2, 2015), 2015.

[4]   J.C. Quinn, R. Davis, The potentials and challenges of algae based biofuels: A review of the techno-economic, life cycle, and resource assessment modeling, Bioresour. Technol. 184 (2015) 444–452. doi:10.1016/j.biortech.2014.10.075.

[5]   D.L. Sills, V. Paramita, M.J. Franke, M.C. Johnson, T.M. Akabas, C.H. Greene, J.W. Tester, Quantitative uncertainty analysis of Life Cycle Assessment for algal biofuel production., Environ. Sci. Technol. 47 (2013) 687–694. doi:10.1021/es3029236.

[6]   C.E. Canter, P. Blowers, R.M. Handler, D.R. Shonnard, Implications of widespread algal biofuels production on macronutrient fertilizer supplies: Nutrient demand and evaluation of potential alternate nutrient sources, Appl. Energy. 143 (2015) 71–80. doi:10.1016/j.apenergy.2014.12.065.

[7]   C.M. Beal, L.N. Gerber, D.L. Sills, M.E. Huntley, S.C. Machesky, M.J. Walsh, J.W. Tester, I. Archibald, J. Granados, C.H. Greene, Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment, Algal Res. 10 (2015) 266–279. doi:10.1016/j.algal.2015.04.017.

[8]   J.J. Mayers, A. Ekman Nilsson, E. Svensson, E. Albers, Integrating Microalgal Production with Industrial Outputs—Reducing Process Inputs and Quantifying the Benefits, Ind. Biotechnol. 12 (2016) ind.2016.0006. doi:10.1089/ind.2016.0006.

[9]   R.M. Handler, D.R. Shonnard, T.N. Kalnes, F.S. Lupton, Life cycle assessment of algal biofuels: Influence of feedstock cultivation systems and conversion platforms, Algal Res. 4 (2014) 105–115. doi:10.1016/j.algal.2013.12.001.

[10] B.K. Shurtz, B. Wood, J.C. Quinn, Nutrient resource requirements for large-scale microalgae biofuel production: Multi-pathway evaluation, Sustain. Energy Technol. Assessments. 19 (2017) 51–58. doi:10.1016/j.seta.2016.11.003.

[11] R.M. Handler, C.E. Canter, T.N. Kalnes, F.S. Lupton, O. Kholiqov, D.R. Shonnard, P. Blowers, Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts, Algal Res. 1 (2012) 83–92. http://www.sciencedirect.com/science/article/pii/S2211926412000069 (accessed 3 December 2013).

[12] S.E. Taelman, S. De Meester, W. Van Dijk, V. da Silva, J. Dewulf, Environmental sustainability analysis of a protein-rich livestock feed ingredient in The Netherlands: Microalgae production versus soybean import, Resour. Conserv. Recycl. 101 (2015) 61–72. doi:10.1016/j.resconrec.2015.05.013.

[13] X. Liu, B. Saydah, P. Eranki, L.M. Colosi, B. Greg Mitchell, J. Rhodes, A.F. Clarens, Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction., Bioresour. Technol. 148 (2013) 163–171. doi:10.1016/j.biortech.2013.08.112.

[14] M.C. Johnson, I. Palou-Rivera, E.D. Frank, Energy consumption during the manufacture of nutrients for algae cultivation, Algal Res. 2 (2013) 426–436. http://www.sciencedirect.com/science/article/pii/S2211926413000854 (accessed 3 December 2013).

[15] S. Wood, A. Cowie, A review of greenhouse gas emission factos for fertiliser production, IEA

Bioenergy Task 38. (2004).

http://t1.netguides.info/downloads/cc/GHG_Emission_Fertilizer_Production_June2004.pdf

(accessed 16 May 2014).

[16]   G. Maggio, G. Cacciola, When will oil, natural gas, and coal peak?, Fuel. 98 (2012) 111–123.

doi:10.1016/j.fuel.2012.03.021.

[17]   D. Cordell, A. Rosemarin, J.J. Schröder, A.L. Smit, Towards global phosphorus security: a

systems framework for phosphorus recovery and reuse options., Chemosphere. 84 (2011) 747–

758. doi:10.1016/j.chemosphere.2011.02.032.

[18]   R. Nehring, Table -7. Average U.S. farm prices of selected fertilizers, 1960-2013, 2013.

[19]   J.J. Mayers, K.J. Flynn, R.J. Shields, Influence of the N: P supply ratio on biomass productivity

and time-resolved changes in elemental and bulk biochemical composition of Nannochloropsis

sp., Bioresour. Technol. 169 (2014) 588–595. doi:10.1016/j.biortech.2014.07.048.

[20]   J. Camacho-Rodríguez, M.C. Cerón-García, C. V González-López, J.M. Fernández-Sevilla, A.

Contreras-Gómez, E. Molina-Grima, A low-cost culture medium for the production of

Nannochloropsis gaditana biomass optimized for aquaculture., Bioresour. Technol. 144 (2013)

57–66. doi:10.1016/j.biortech.2013.06.083.

[21]   T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: Status and

prospects, Renew. Sustain. Energy Rev. 19 (2013) 360–369. doi:10.1016/j.rser.2012.11.030.

[22]   P. Biller, A.B. Ross, S.C. Skill, A. Lea-Langton, B. Balasundaram, C. Hall, R. Riley, C.A.

Llewellyn, Nutrient recycling of aqueous phase for microalgae cultivation from the

hydrothermal liquefaction process, Algal Res. 1 (2012) 70–76.

doi:10.1016/j.algal.2012.02.002.

[23]   J. Gasperi, S. Garnaud, V. Rocher, R. Moilleron, Priority pollutants in wastewater and

combined sewer overflow., Sci. Total Environ. 407 (2008) 263–72.

doi:10.1016/j.scitotenv.2008.08.015.

[24]   M. van der Spiegel, M.Y. Noordam, H.J. van der Fels-Klerx, Safety of Novel Protein Sources

(Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their

Application in Food and Feed Production, Compr. Rev. Food Sci. Food Saf. 12 (2013) 662–

678. doi:10.1111/1541-4337.12032.

[25]   P. Rzymski, P. Niedzielski, N. Kaczmarek, T. Jurczak, P. Klimaszyk, The multidisciplinary

approach to safety and toxicity assessment of microalgae-based food supplements following

clinical cases of poisoning, Harmful Algae. 46 (2015) 34–42. doi:10.1016/j.hal.2015.05.003.

[26]   C.J. Banks, A.M. Salter, S. Heaven, K. Riley, Energetic and environmental benefits of co-

digestion of food waste and cattle slurry: A preliminary assessment, Resour. Conserv. Recycl.

56 (2011) 71–79. doi:10.1016/j.resconrec.2011.09.006.

[27]   WRAP, Market expectations and requirements for digestate, 2013.

[28]   A. Akhiar, A. Battimelli, M. Torrijos, H. Carrere, Comprehensive characterization of the liquid

fraction of digestates from full-scale anaerobic co-digestion, Waste Manag. 59 (2017) 118–

128. doi:10.1016/j.wasman.2016.11.005.

[29]   N. Gregson, M. Crang, S. Fuller, H. Holmes, Interrogating the circular economy: the moral

economy of resource recovery in the EU, Econ. Soc. 44 (2015) 218–243.

doi:10.1080/03085147.2015.1013353.

[30]   DEFRA, DECC, Anaerobic digestion strategy and action plan, 2011.

https://www.gov.uk/government/publications/anaerobic-digestion-strategy-and-action-plan

(accessed 19 May 2014).

[31]   European Commission, The Roadmap to a Resource Efficient Europe (COM 2011 - 571),

2011. (n.d.). http://ec.europa.eu/environment/resource_efficiency/about/roadmap/index_en.htm

(accessed 2 June 2017).

[32]   O. Fenton, Ó. Daire, Agricultural nutrient surpluses as potential input sources to grow third

generation biomass (microalgae): A review, Algal Res. 1 (2012) 49–56.

doi:10.1016/j.algal.2012.03.003.

[33]   A. Xia, J.D. Murphy, Microalgal Cultivation in Treating Liquid Digestate from Biogas

Systems, Trends Biotechnol. 34 (2016) 264–275. doi:10.1016/j.tibtech.2015.12.010.

[34]   Environment Agency, WRAP, Quality Protocol: Anaerobic digestate, 2014.

hhttp://www.biofertiliser.org.uk/pdf/Anaerobic-Digestion-Quality-Protocol.pdf (accessed 19

May 2014).

[35]   L. Wang, Y. Li, P. Chen, M. Min, Y. Chen, J. Zhu, R.R. Ruan, Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp., Bioresour. Technol. 101 (2010) 2623–8. doi:10.1016/j.biortech.2009.10.062.

[36]   T. Cai, S.Y. Park, R. Racharaks, Y. Li, Cultivation of Nannochloropsis salina using anaerobic digestion effluent as a nutrient source for biofuel production, Appl. Energy. 108 (2013) 486–492. doi:10.1016/j.apenergy.2013.03.056.

[37]   J.P. Sheets, X. Ge, S.Y. Park, Y. Li, Effect of outdoor conditions on Nannochloropsis salina cultivation in artificial seawater using nutrients from anaerobic digestion effluent, Bioresour. Technol. 152 (2013) 154–161. http://www.sciencedirect.com/science/article/pii/S0960852413017021 (accessed 9 November 2013).

[38]   R.A. Andersen, Algal Cultering Techniques, Elsevier Science, Oxford, UK, 2005.

[39]   J.J. Mayers, K.J. Flynn, R.J. Shields, Rapid determination of bulk microalgal biochemical composition by Fourier-Transform Infrared spectroscopy, Bioresour. Technol. 148 (2013) 215–220. doi:10.1016/j.biortech.2013.08.133.

[40]   J.D.H. Strickland, T.R. Parsons, Determination of reactive phosphorus, in: J.D.H. Strickland, T.R. Parsons (Eds.), A Pract. Handb. Sewater Anal., 2nd Ed., Fishers Research Board Of Canada, Ottawa, 1968: pp. 49–56. http://www.scopus.com/inward/record.url?eid=2-s2.0-77951257833&partnerID=tZOtx3y1.

[41]   L.M.L. Laurens, M. Quinn, S. Van Wychen, D.W. Templeton, E.J. Wolfrum, Accurate and reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification., Anal. Bioanal. Chem. 403 (2012) 167–78. doi:10.1007/s00216-012-5814-0.

[42]   B.P. Weidema, C. Bauer, R. Hischier, C. Mutel, T. Nemecek, J. Reinhard, C.O. Vadenbo, G. Wernet, Data quality guideline for the ecoinvent database version 3, 3 (2013) 169. http://www.ecoinvent.org/database/methodology-of-ecoinvent-3/methodology-of-ecoinvent-3.html.

[43]   B. Kim, H. Im, J.W. Lee, In situ transesterification of highly wet microalgae using hydrochloric acid, 2015. doi:10.1016/j.biortech.2015.02.092.

[44] A.C. Redfield, The biological control of chemical factors in the environment, Am. Sci. 46 (1958) 205–21. http://ebme.marine.rutgers.edu/HistoryEarthSystems/HistEarthSystems_Fall2008/Week4b/Redfield_AmSci_1958.pdf (accessed 2 August 2014).

[45] L. Jiang, S. Luo, X. Fan, Z. Yang, R. Guo, Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO2, Appl. Energy. 88 (2011) 3336–3341. doi:10.1016/j.apenergy.2011.03.043.

[46] J. Van Wagenen, T.W. Miller, S. Hobbs, P. Hook, B. Crowe, M. Huesemann, Effects of light and temperature on fatty acid production in Nannochloropsis salina, Energies. 5 (2012) 731–740. doi:10.3390/en5030731.

[47] A. San Pedro, C. V González-López, F.G. Acién, E. Molina-Grima, Outdoor pilot-scale production of Nannochloropsis gaditana: influence of culture parameters and lipid production rates in tubular photobioreactors., Bioresour. Technol. 169 (2014) 667–76. doi:10.1016/j.biortech.2014.07.052.

[48] S.G. Shin, G. Han, J. Lee, K. Cho, E.-J. Jeon, C. Lee, S. Hwang, Characterization of food waste-recycling wastewater as biogas feedstock, Bioresour. Technol. (2015). doi:10.1016/j.biortech.2015.07.089.

[49] G. Breuer, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains., Bioresour. Technol. 124 (2012) 217–26. doi:10.1016/j.biortech.2012.08.003.

[50] C. Adams, V. Godfrey, B. Wahlen, L. Seefeldt, B. Bugbee, Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in oleaginous green microalgae., Bioresour. Technol. 131 (2013) 188–194. doi:10.1016/j.biortech.2012.12.143.

[51] J.J. Mayers, K.J. Flynn, R.J. Shields, Influence of the N:P supply ratio on biomass productivity and time-resolved changes in elemental and bulk biochemical composition of Nannochloropsis sp., Bioresour. Technol. 169 (2014) 588–595. doi:10.1016/j.biortech.2014.07.048.

[52] H. Campos, W.J. Boeing, B.N. Dungan, T. Schaub, Cultivating the marine microalga Nannochloropsis salina under various nitrogen sources: Effect on biovolume yields, lipid

content and composition, and invasive organisms, Biomass and Bioenergy. 66 (2014) 301–307. doi:10.1016/j.biombioe.2014.04.005.

[53]   Y.S. Hii, C.L. Soo, T.S. Chuah, Interactive effect of ammonia and nitrate on the nitrogen uptake by Nannochloropsis sp., 6 (2011) 60–68.

[54]   M. Podevin, D. De Francisci, S.L. Holdt, I. Angelidaki, Effect of nitrogen source and acclimatization on specific growth rates of microalgae determined by a high-throughput in vivo microplate autofluorescence method, J. Appl. Phycol. 27 (2015) 1415–1423. doi:10.1007/s10811-014-0468-2.

[55]   E. Sforza, R. Cipriani, T. Morosinotto, A. Bertucco, G.M. Giacometti, Excess CO2 supply inhibits mixotrophic growth of Chlorella protothecoides and Nannochloropsis salina., Bioresour. Technol. 104 (2012) 523–9. doi:10.1016/j.biortech.2011.10.025.

[56]   J. Gutierrez, T.A. Kwan, J.B. Zimmerman, J. Peccia, Ammonia inhibition in oleaginous microalgae, Algal Res. 19 (2016) 123–127. doi:10.1016/j.algal.2016.07.016.

[57]   R.W. Davis, A.J. Siccardi, N.D. Huysman, N.B. Wyatt, J.C. Hewson, T.W. Lane, Growth of mono- and mixed cultures of Nannochloropsis salina and Phaeodactylum tricornutum on struvite as a nutrient source, Bioresour. Technol. 198 (2015) 577–585. doi:10.1016/j.biortech.2015.09.070.

[58]   J. Park, H.-F. Jin, B.-R. Lim, K.-Y. Park, K. Lee, Ammonia removal from anaerobic digestion effluent of livestock waste using green alga Scenedesmus sp., Bioresour. Technol. 101 (2010) 8649–57. doi:10.1016/j.biortech.2010.06.142.

[59]   C. Adams, B. Bugbee, Nitrogen retention and partitioning at the initiation of lipid accumulation in nitrogen-deficient algae, J. Phycol. 50 (2014) 356–365. doi:10.1111/jpy.12167.

[60]   T.A. Beacham, C. Bradley, D.A. White, P. Bond, S.T. Ali, Lipid productivity and cell wall ultrastructure of six strains of Nannochloropsis: Implications for biofuel production and downstream processing, Algal Res. 6 (2014) 64–69. doi:10.1016/j.algal.2014.09.003.

[61]   D. Simionato, M.A. Block, N. La Rocca, J. Jouhet, E. Maréchal, G. Finazzi, T. Morosinotto, The response of Nannochloropsis gaditana to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the

photosynthetic apparatus., Eukaryot. Cell. 12 (2013) 665–76. doi:10.1128/EC.00363-12.

[62]   X. Johnson, J. Alric, Central carbon metabolism and electron transport in Chlamydomonas

reinhardtii: metabolic constraints for carbon partitioning between oil and starch., Eukaryot.

Cell. 12 (2013) 776–93. doi:10.1128/EC.00318-12.

[63]   B. Liu, C. Benning, Lipid metabolism in microalgae distinguishes itself, Curr. Opin.

Biotechnol. 24 (2013) 300–9. doi:10.1016/j.copbio.2012.08.008.

[64]   A.J. Klok, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, Edible oils from

microalgae: insights in TAG accumulation., Trends Biotechnol. 32 (2014) 521–528.

doi:10.1016/j.tibtech.2014.07.004.

[65]   Y. Li, D. Han, K. Yoon, S. Zhu, M. Sommerfeld, Q. Hu, Molecular and Cellular Mechanisms

for Lipid Synthesis and Accumulation in Microalgae: Biotechnological Implications, in: A.

Richmond, Q. Hu (Eds.), Handb. Microalgal Cult. Biotechnol. Appl. Phycol., 2nd Editio, John

Wiley & Sons, Ltd, Oxford, UK, 2013: pp. 545–565. doi:10.1002/9781118567166.

[66]   J.A. Raven, The cost of photoinhibition, Physiol. Plant. 142 (2011) 87–104.

doi:10.1111/j.1399-3054.2011.01465.x.

[67]   P. Geigenberger, Redox regulation of carbon storage and partitioning in response to light and

sugars, J. Exp. Bot. 56 (2005) 1469–1479. doi:10.1093/jxb/eri178.

[68]   J.P. Sheets, X. Ge, S.Y. Park, Y. Li, Effect of outdoor conditions on Nannochloropsis salina

cultivation in artificial seawater using nutrients from anaerobic digestion effluent, Bioresour.

Technol. 152 (2014) 154–161. doi:10.1016/j.biortech.2013.10.115.

[69]   H. Hu, K. Gao, Response of growth and fatty acid compositions of Nannochloropsis sp. to

environmental factors under elevated CO2 concentration., Biotechnol. Lett. 28 (2006) 987–

992. doi:10.1007/s10529-006-9026-6.

[70]   P. Das, W. Lei, S.S. Aziz, J.P. Obbard, Enhanced algae growth in both phototrophic and

mixotrophic culture under blue light, Bioresour. Technol. 102 (2011) 3883–3887.

doi:10.1016/j.biortech.2010.11.102.

[71]   J. Camacho-Rodríguez, M.C. Cerón-García, J.M. Fernández-Sevilla, E. Molina-Grima, Genetic

algorithm for the medium optimization of the microalga Nannochloropsis gaditana cultured to

aquaculture., Bioresour. Technol. 177 (2015) 102–9. doi:10.1016/j.biortech.2014.11.057.

[72] R. Racharaks, X. Ge, Y. Li, Cultivation of marine microalgae using shale gas flowback water and anaerobic digestion effluent as the cultivation medium, Bioresour. Technol. 191 (2015) 146–156. doi:10.1016/j.biortech.2015.04.065.

[73] H.B. Bucy, M.E. Baumgardner, A.J. Marchese, Chemical and physical properties of algal methyl ester biodiesel containing varying levels of methyl eicosapentaenoate and methyl docosahexaenoate, Algal Res. 1 (2012) 57–69. doi:10.1016/j.algal.2012.02.001.

[74] M. Islam, M. Magnusson, R. Brown, G. Ayoko, M. Nabi, K. Heimann, Microalgal Species Selection for Biodiesel Production Based on Fuel Properties Derived from Fatty Acid Profiles, Energies. 6 (2013) 5676–5702. doi:10.3390/en6115676.

[75] R. Geider, J. La Roche, Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis, Eur. J. Phycol. 37 (2002) 1–17. doi:10.1017/S0967026201003456.

[76] P. Collet, A. Hélias, L. Lardon, J.-P. Steyer, O. Bernard, Recommendations for Life Cycle Assessment of algal fuels, Appl. Energy. 154 (2015) 1089–1102. doi:10.1016/j.apenergy.2015.03.056.

[77] F. Delrue, P.-A. Setier, C. Sahut, L. Cournac, A. Roubaud, G. Peltier, A.-K. Froment, An economic, sustainability, and energetic model of biodiesel production from microalgae., Bioresour. Technol. 111 (2012) 191–200. doi:10.1016/j.biortech.2012.02.020.

[78] J.N. Rogers, J.N. Rosenberg, B.J. Guzman, V.H. Oh, L.E. Mimbela, A. Ghassemi, M.J. Betenbaugh, G.A. Oyler, M.D. Donohue, A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales, Algal Res. 4 (2014) 76–88. doi:10.1016/j.algal.2013.11.007.

[79] M.E. Huntley, Z.I. Johnson, S.L. Brown, D.L. Sills, L. Gerber, I. Archibald, S.C. Machesky, J. Granados, C. Beal, C.H. Greene, Demonstrated large-scale production of marine microalgae for fuels and feed, Algal Res. 10 (2015) 249–265. doi:10.1016/j.algal.2015.04.016.

[80] D.L. Medeiros, E.A. Sales, A. Kiperstok, Energy production from microalgae biomass: carbon footprint and energy balance, J. Clean. Prod. 96 (2014) 493–500. doi:10.1016/j.jclepro.2014.07.038.

[81]   A.J. Dassey, S.G. Hall, C.S. Theegala, An analysis of energy consumption for algal biodiesel

        production: Comparing the literature with current estimates, Algal Res. 4 (2014) 89–95.

        doi:10.1016/j.algal.2013.12.006.

[82]   N.-H. Norsker, M.J. Barbosa, M.H. Vermuë, R.H. Wijffels, Microalgal production — A close

        look at the economics, Biotechnol. Adv. 29 (2011) 24–27.

        doi:10.1016/j.biotechadv.2010.08.005.

[83]   M.R. Tredici, L. Rodolfi, N. Biondi, N. Bassi, G. Sampietro, Techno-economic analysis of

        microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant, Algal Res. 19

        (2016) 253–263. doi:10.1016/j.algal.2016.09.005.

[84]   J. Hoffman, R.C. Pate, T. Drennen, J.C. Quinn, Techno-economic assessment of open

        microalgae production systems, Algal Res. 23 (2017) 51–57. doi:10.1016/j.algal.2017.01.005.

[85]   Alternative Fuels Data Center: Fuel Prices, (n.d.).

        http://www.afdc.energy.gov/fuels/prices.html (accessed 13 January 2017).

[86]   J.A. Posada, L.B. Brentner, A. Ramirez, M.K. Patel, Conceptual design of sustainable

        integrated microalgae biorefineries: Parametric analysis of energy use, greenhouse gas

        emissions and techno-economics, Algal Res. 17 (2016) 113–131.

        doi:10.1016/j.algal.2016.04.022.

[87]   R. Davis, D. Fishman, E.D. Frank, M.S. Wigmosta, A. Aden, A.M. Coleman, P.T. Pienkos,

        R.J. Skaggs, E.R. Venteris, M.Q. Wang, Renewable Diesel from Algal Lipids: An Integrated

        Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model, Golden, CO

        (United States), 2012. doi:10.2172/1044475.

[88]   R. Slade, A. Bauen, Micro-algae cultivation for biofuels: Cost, energy balance, environmental

        impacts and future prospects, Biomass and Bioenergy. 53 (2013) 29–38.

        doi:10.1016/j.biombioe.2012.12.019.