Algal Research 6 (2014) 22–31

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Pre- and post-harvest treatment of macroalgae to improve the quality of feedstock for hydrothermal liquefaction

N. Neveux [a,*], A.K.L. Yuen [b], C. Jazrawi [c], Y. He [c], M. Magnusson [a], B.S. Haynes [c], A.F. Masters [b], A. Montoya [c], N.A. Paul [a], T. Maschmeyer [b], R. de Nys [a]

[a] MACRO, the Centre for Macroalgal Resources & Biotechnology, James Cook University Townsville, QLD 4811, Australia
[b] School of Chemistry, Building F11, The University of Sydney, NSW 2006, Australia
[c] School of Chemical & Biomolecular Engineering, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 4 August 2014
Accepted 16 August 2014
Available online 7 September 2014

Keywords:
Algae
Cultivation
Nitrogen
Hydrothermal liquefaction
Biocrude
Biofuel

ABSTRACT

Three species of macroalgae were treated with the aim of reducing nitrogen, sulfur and ash within the biomass prior to hydrothermal processing. The treatments were the nutrient starvation of cultures and post-harvest washing of biomass in freshwater. Subsequently, hydrothermal liquefaction (HTL) of macroalgae was carried out in a batch reactor heated for 8 min with a maximum temperature of 345 °C. Nutrient starvation effectively reduced nitrogen and sulfur levels within the biomass, which led to a reduction in nitrogen by 51–59 wt.% and sulfur by 64–88 wt.% within the biocrude. The yield of biocrude was highest for *Derbesia* at 38.6–41.7 wt.% and *Oedogonium* at 35.6–38.8 wt.% when not starved, but was reduced by up to 19 wt.% when the biomass was starved. The washing of biomass consistently reduced the ash content for all species by 7–83 wt.%. The removal of ash affected neither the quality nor the quantity of biocrude produced. The two treatments demonstrate that macroalgal biomass can be effectively manipulated in the production process to modify the composition of the feedstock and, consequently, improve the quality of biocrude. Additionally, reducing the ash content of biomass minimizes its potential impact on HTL processing equipment.

1. Introduction

Macroalgal biomass is a diverse and abundant resource for the innovative production of renewable liquid fuels and chemicals [1–4]. Macroalgae are often highly productive on an areal basis, are simple to harvest and process, and can be produced on non-arable land, as well as in freshwater and in the sea. These advantages combine ideally with the efficiencies of hydrothermal liquefaction (HTL), a thermochemical process using hot compressed water at conditions approaching the critical point of water (374 °C; 22.1 MPa) to decompose wet biomass to a liquid biocrude [5,6]. The thermochemical decomposition of biomass relies on the unique properties of water at these subcritical conditions, where it acts simultaneously as a solvent (similar to acetone), reactant, and both acid and base catalyst, due to its increased auto-ionization. Elevated temperatures and pressures reduce the density, polarity and relative permittivity/dielectric constant of water, resulting in the hydrolysis and dissolution of solid biomass [7]. A complex network of cascading reactions involving the newly liberated low molecular weight hydrocarbons leads to the formation of an oily biocrude, gases (principally CO_2), water-soluble chemicals and insoluble residues (biochar).

Biocrude produced through the HTL of algae has a high energy density that is 70–95% of that of petroleum crude [8]. The difference in energy is due to the presence of heteroatoms (O, N, S), derived mainly from the protein and carbohydrate fractions of the biomass, accounting for 10–20% of the mass of algal biocrude [4,9,10]. The reduction or removal of these heteroatoms within the biocrude prior to upgrading into liquid hydrocarbon 'drop-in' fuel or into feedstock for the production of industrial chemicals would be highly beneficial [11]. Previous studies have demonstrated that it is possible to reduce the content of O, N, and S within the biocrude through catalytic hydrotreating, although this treatment requires substantial H_2 and energy inputs [12–14]. Other studies have demonstrated that oxygen and trace metals can be efficiently reduced within the biocrude through thermal treatment, but the nitrogen content is not improved by such treatment [15,16]. The presence of nitrogenous and sulfurous compounds in the biocrude is particularly detrimental as nitrogen can poison the active sites of catalysts used in conventional refining and both elements can participate in the formation of harmful nitrogen and sulfur oxide emissions during combustion [17]. Another issue for HTL processing of macroalgae – and specifically marine macroalgae – is the presence of inorganic compounds (ash as silicates, hydroxides, metal oxides, halides, carbonates, and sulfates with alkali-metal counterions) which can precipitate and deposit on reactor walls, thereby blocking reactors, and in the case of halides in particular, cause corrosion and the

* Corresponding author. Tel.: +61 7 4781 4778; fax: +61 7 4781 4585.
E-mail address: nicolas.neveux@my.jcu.edu.au (N. Neveux).

http://dx.doi.org/10.1016/j.algal.2014.08.008
2211-9264/

degradation of the stainless steel reaction vessels [7,17]. Trace amounts of metals (i.e. Fe, Mg, Zn, Ni) can also be transferred to the biocrude and become a significant challenge for upgrading in conventional refinery units [16].

One route to circumvent both the extensive treatment of the biocrude and the HTL processing issues arising from nitrogen, sulfur and ash in macroalgae, would be to reduce these components in the feedstock prior to hydrothermal upgrading. Nitrogen is a key element in algal metabolism that is essential for the formation of proteins and chlorophyll, and therefore photosynthesis [18]. However, the content of nitrogen is highly variable in macroalgae and can be reduced through a starvation process, where the biomass continues to grow in a low nitrogen environment, thereby diluting the internal nitrogen pool to a minimum [19]. Sulfur also has a pivotal role in algal cell physiology and homeostasis through its role in the formation of dimethylsulfoniopropionate (DMSP), sulfolipids and various amino acids [20]. The treatment of macroalgae through nutrient starvation and washing may result in a decreased content of sulfur within the biomass. Consequently, the HTL processing of this biomass may improve the quality of biocrude. Similarly, the minimization of the ash content of the biomass through the removal of salts can be expected to reduce the mechanical demands on HTL processing equipment. To our knowledge this is the first report on the combined effects that metabolic manipulation of the content of nitrogen, sulfur and ash in the biomass have on the yield and quality of biocrude. This approach, which focuses on tailoring the algal feedstock for a specific purpose, is a critical first step in the delivery of an improved biocrude that minimizes hydrotreating requirements (particularly hydrodenitrogenation and hydrodesulfurization) for the production of a fully fungible biofuel.

Therefore, the aim of this study was to assess if the manipulation of freshwater and marine macroalgae through nutrient starvation, and the post-harvest washing of biomass, would reduce the content of nitrogen, sulfur and ash prior to HTL processing, and whether these changes would be carried through the HTL process, affording a desirable biocrude product. Firstly the effects of starvation and washing on the composition of biomass were evaluated. Subsequently, the yield and elemental composition of biocrude and HTL co-products produced from algae subject to combinations of starvation and washing were assessed. Finally, the variation in the content of carbon in each of the treated algal feedstocks was correlated with the yield of biocrude.

2. Materials and methods

2.1. Culturing of macroalgae

Three species of green macroalgae (Chlorophyta) were selected based on their high productivity in land-based culture and high conversion yield to biocrude [3,4]. Samples were harvested in November 2012 from stock cultures held in outdoor tanks at James Cook University, Townsville. Species were the marine macroalgae *Derbesia tenuissima* [21] and *Ulva ohnoi* [22] and the freshwater macroalga *Oedogonium* sp. [23]. Macroalgae were placed in 50 L cylindrical tanks in an outdoor system to be cultured for 36 days. Biomass was initially stocked at 2 g/L fresh weight (fw) for marine species and 0.5 g/L (fw) for the freshwater species based on individual stocking density trials [19,21,23]. Macroalgae were cultivated in a batch culture system, described in detail previously [3]. Biomass was harvested every 6 days (6 cycles of 6 days each in total) using a net (2 mm screen), spun to a constant fresh weight, weighed and subsequently re-stocked at initial stocking densities for a new cycle. Excess biomass was discarded. Water in the batch tanks was entirely renewed every 6 days using saltwater (35 g/L of dissolved salts) for marine species and dechlorinated freshwater (0–1 g/L of dissolved salts) for the freshwater species. Environmental conditions were monitored daily and adjusted accordingly. Salinity for marine species was adjusted daily by adding dechlorinated freshwater to compensate for evaporation. Salinity in freshwater cultures was stable for the duration of the experiment. The pH in batch cultures varied naturally between 8.3 (sunrise) to 9.4 (sunset) for marine species and between 8.4 (sunrise) to 10.3 (sunset) for the freshwater species. Culture tanks were placed inside a larger holding tank for temperature control at 25 °C with a continuous flow of water. Light was monitored hourly using a photosynthetically active radiation data logger (Li-1400; LI-COR, Inc., Lincoln, Nebraska, USA) adjacent to the tanks for the duration of the experiment. Total photons received over each 6-day culture cycle ranged from 301 to 349 mol photons/m^2.

2.2. Nutrient starvation and washing treatments

A schematic diagram of the culture method and treatments is shown in Fig. 1. The initial growth phase (18 days, three 6-day culture cycles) provided nutrients in excess until stable productivity was reached, using f/2 medium [24] for marine species and f/4 medium for freshwater species. During this phase, eight replicates of each of the three species were cultured (N+; n = 8). The second phase, or starvation phase (also 18 days, three 6-day culture cycles), consisted of removing the nutrient supply from half of the culture replicates (N−; n = 4), while the other half remained supplied with the same nutrients as in the growth phase (N+; n = 4). After a total of 36 days of culture, all biomass in each tank was harvested, spun and weighed.

Then, macroalgae were further treated to quantify the effect of washing on the ash (dry inorganic) content of biomass. The biomass from each replicate of each species both not starved (N+; n = 4) and starved (N−; n = 4) was divided in equal amounts. Half of the biomass then remained unwashed (A+; n = 4), while the other half of the biomass was washed (A−; n = 4) three times for 1 min by immersing the biomass in town water (~3 L/100 g of algae), stirring and draining the water at each wash. As a result of the starvation and washing procedures, four treatment combinations existed for each species denominated N+/A+, N+/A−, N−/A+, and N−/A−.

2.3. Biomass characterization

A sub-sample of each replicate of each of the treatments was weighed (fw) and oven-dried for 12 h at 60 °C, placed in a desiccator for 30 min at room temperature to reach stable moisture content, and weighed again (dw) to determine the fresh to dry weight ratio (fw: dw). The remaining biomass was freeze-dried, ground to a mean particle size <500 μm, placed in a desiccator for 30 min and then stored in air-tight vials under refrigeration until further analyses. Powdered macroalgae (dw) were used for ash, moisture, lipid and ultimate analyses (see Neveux et al. [3] for details). Protein content was determined using the nitrogen to protein conversion factors of 4.8 for *Derbesia*, 4.6 for *Ulva* and 4.7 for *Oedogonium* [3]. Carbohydrates were determined by difference by subtracting the sum of ash, moisture, lipid and protein weight percentages from 100%.

2.4. Hydrothermal liquefaction

Hydrothermal liquefaction (HTL) of the three macroalgae was performed on each replicate (n = 4) of the four treatments, for a total of 48 runs. HTL was performed using a custom-built stainless steel batch reactor system as described in detail in Neveux et al. [4]. A slurry (6.6 wt.% solids) composed of 2 g of algae powder and 28 mL of distilled water was loaded in the 35 mL (internal volume) reactor tube for each run. The reactor was subsequently fitted with a gasket and attached to a pressure-head, able to handle pressure of up to 25 MPa at 350 °C. The reactor was purged three times at room temperature with N_2 to remove excess oxygen, after which it was pressurized to 7 MPa with N_2. The reactor was then immersed in a fluidized sand bath set to 350 °C to initiate the reaction. The temperature in the reactor was monitored via a thermocouple located inside the reactor above the slurry and the pressure was monitored externally. Typically, the internal temperature

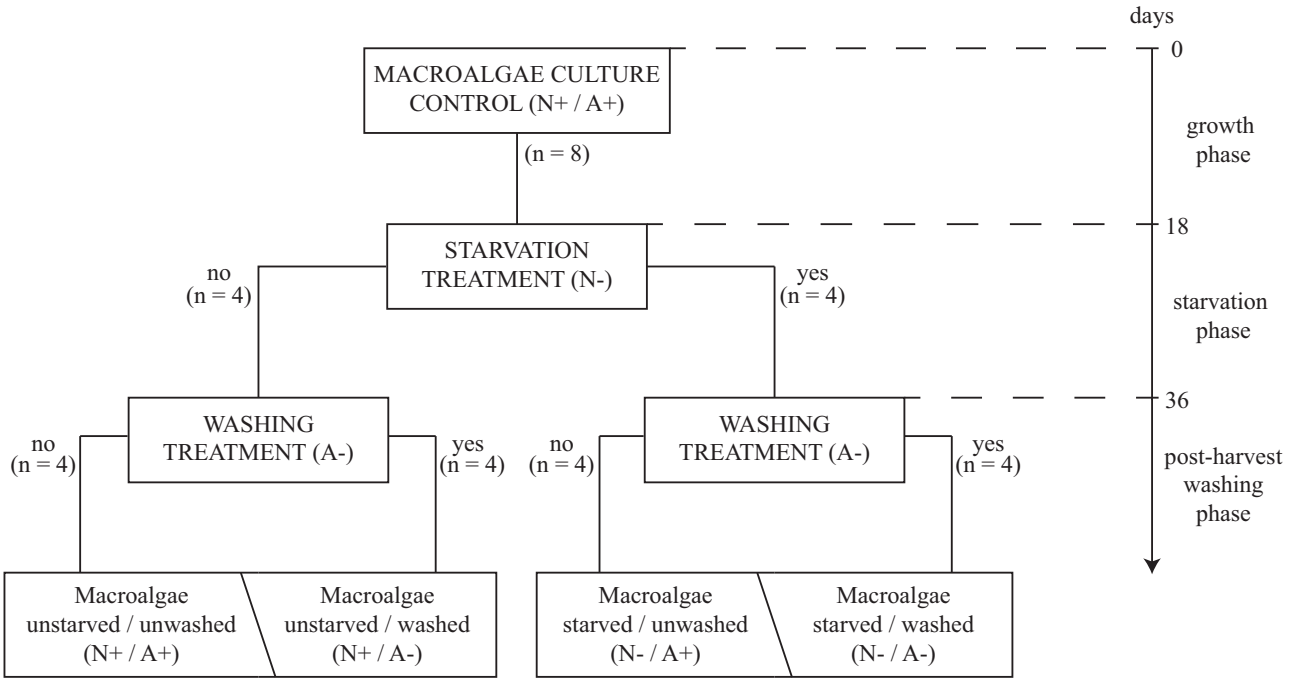

Fig. 1. Schematic diagram of the experimental set-up.

rose on average to 252 °C (9.6 MPa) within 1 min, 309 °C (11.5 MPa) within 2 min and 328 °C (12.9 MPa) within 3 min of reaction time. Internal reaction temperatures of between 330 °C and 345 °C (maximum temperatures and pressures, 14–17 MPa) were maintained for a further 5 min (total of 8 min reaction time) before the reactor was quenched in an ice/water bath for 1 min to cool the reactor and contents to room temperature.

2.5. Product separation and analysis

The gas produced by the HTL of biomass was vented inside a fume hood immediately following the reaction quench and prior to disassembly, and was therefore not sampled. In contrast, the condensed phases (biocrude, biochar and aqueous phase) were separated and analyzed. The reaction mixture (excluding the gaseous product) was diluted with dichloromethane (DCM) and distilled water (25 mL each) and suction filtered over a Whatman grade 2 paper. The residue was further washed with DCM and water, followed by drying at 80 °C for 12 h to afford a dry solid fraction (biochar). The biphasic filtrate was transferred to a separation funnel to isolate the biocrude phase (dissolved in DCM) from the aqueous phase. The aqueous phase was further washed twice with 25 mL of DCM. The pooled organic phase was subsequently concentrated under reduced pressure in a rotary evaporator and dried at 50 °C and 23 mbar to give a dark brown oil (biocrude). Biocrude and biochar yields were calculated separately on a dry ash-free basis (afdw) using Eq. (1):

$$Y_{PRODUCT} = W_{PRODUCT}/W_{BIOMASS} \times 100\% \quad (1)$$

, where $Y_{PRODUCT}$ is the yield of biocrude or biochar (afdw %) and $W_{PRODUCT}$ is the mass of biocrude or biochar (g). $W_{BIOMASS}$ is the organic mass of algae processed (g) and was calculated by subtraction of the sum of ash (g) and moisture (g) from the total mass of the algae.

The ultimate analysis of biocrude and biochar was performed externally (OEA Laboratory Ltd., Callington, Cornwall, UK). The aqueous phase (post-separation) was transferred to a volumetric flask and made up to 100 mL using distilled water for subsequent quantification of total organic carbon, inorganic carbon and nitrogen (TropWATER Analytical Services, James Cook University, Townsville, Queensland, Australia).

2.6. Chemical energy recovery and mass balance

The chemical energy recovery (ER) was calculated for the biocrude and biochar products according to Eq. (2):

$$ER = (HHV_{PRODUCT} \times W_{PRODUCT})/(HHV_{FEEDSTOCK} \times W_{FEEDSTOCK}) \times 100\% \quad (2)$$

, where ER is the energy recovery of the biocrude or biochar (%), $HHV_{PRODUCT}$ is the biocrude or biochar higher heating value (MJ/kg), $W_{PRODUCT}$ is the mass of biocrude or biochar (g), $HHV_{FEEDSTOCK}$ is the macroalgae higher heating value (MJ/kg) and $W_{FEEDSTOCK}$ is the mass of macroalgae processed (g, dw). The higher heating value (HHV) of biomass, biocrude and biochar was calculated with the unified correlation proposed by Channiwala and Parikh [25].

Eq. (2) was also used to determine the mass balance in product streams, specifically the mass of elements C, H, O, N, and S recovered in biocrude and biochar, by substituting HTL products and feedstock HHV with elemental contents. The remaining elemental fractions allowed an estimation of the energy and mass partitioned to the combined aqueous and gas phases, and losses.

2.7. Statistical analysis

Factorial analyses of variance (two- and three-way ANOVAs) were performed to assess the main effects and interactions between starvation and washing treatments on the composition and productivity of biomass, and on the yield of biocrude, using STATISTICA 10 software (StatSoft Inc., Tulsa, Oklahoma, USA). Residual plots and normality tests were used to ensure that ANOVA assumptions were met. Significant differences between the treatments are reported at the $\alpha = 0.05$ level of significance. As there were significant interactions in each ANOVA (see the Results section), no formal post-hoc comparisons were made between treatments for each main effect. The productivity of the biomass was only formally analyzed for the last culture cycle (cycle 6, 3rd cycle of starvation), since this was the biomass used for

all subsequent biochemical analyses and HTL. The elemental composition of HTL products was not analyzed formally as the individual replicates of biocrude, biochar and aqueous phases were combined for each treatment prior to elemental analysis. All results are reported on a dry weight basis unless stated otherwise.

3. Results

3.1. Biomass productivity

The productivity of macroalgae cultured for 6 cycles of 6 days is presented in Fig. 2 with the nutrient starvation treatment starting after 3 cycles. All cultures that were continuously provided with nutrients (N+, cycles 1 to 6) had a stable productivity over the entire culture period. *Ulva* had the highest productivity with an average (±1SE) of 21.6 ± 0.9 g/m^2/d dw over the 6 culture cycles, followed by *Derbesia* at 12.7 ± 0.5 g/m^2/d dw and *Oedogonium* at 9.7 ± 0.2 g/m^2/d dw. Predictably, the nutrient starvation phase (N−, cycles 4 to 6) resulted in a consistent and in some cases dramatic decrease in productivity (cycle 6, ANOVA, $F_{1,18} = 476.7$, $P < 0.05$). After one cycle of starvation (cycle 4), the productivity of the marine species decreased by more than 50% to 7.0 g/m^2/d dw for *Ulva*, and 5.7 g/m^2/d dw for *Derbesia*. The subsequent cycles of starvation resulted in further decreases in productivity for *Ulva* and *Derbesia* to 0.5 and 0.9 g/m^2/d dw respectively. In the third cycle of starvation (cycle 6) there was no further increase in biomass, and, therefore, no further dilution of the internal nitrogen pool. Consequently, the cultivation phase was completed at this stage. Interestingly, the productivity of freshwater *Oedogonium* remained stable in the first cycle of starvation (cycle 4), maintaining growth at 10.6 g/m^2/d dw without the addition of nutrients, with a subsequent decrease in productivity to 3.7 g/m^2/d dw in the final culture cycle (cycle 6). A significant interaction effect between species and the starvation treatment (ANOVA, $F_{1,18} = 46.4$, $P < 0.05$) was the result of the marine species having higher growth rates under nutrient supply and the freshwater species being less affected by nutrient starvation.

3.2. Feedstock characterization

Table 1 shows the proximate, biochemical, ultimate and elemental analyses for each of the macroalgae species subjected to the various growth (N+, N−) and washing (A+, A−) treatments. As hypothesized, nutrient starvation (N−) had a significant effect on the organic profile of the biomass, primarily the protein content, with an average reduction in protein (±1SE) of 73 ± 3% for *Derbesia*, 75 ± 2% for *Ulva* and 71 ± 4% for *Oedogonium*, compared with biomass that was not starved of nutrients (N+) (ANOVA, $F_{1,36} = 589.4$, $P < 0.05$). This reduction is highlighted in Fig. 3 with a substantial decrease of the nitrogen content of biomass resulting from nutrient starvation. This treatment also had a significant effect on lipid content with an average reduction in lipid of 53 ± 2% for *Derbesia*, 36 ± 10% for *Ulva* and 58 ± 4% for *Oedogonium* (ANOVA, $F_{1,36} = 220.3$, $P < 0.05$). Consequently, there was an increase in carbohydrate content of 91 ± 8% for *Derbesia*, 36 ± 4% for *Ulva* and 34 ± 5% for *Oedogonium* (ANOVA, $F_{1,36} = 539.6$, $P < 0.05$). This modification of the organic profile was also manifested at the elemental level, with an average reduction in the carbon and energy contents of 7 ± 1% and 12 ± 1% respectively, across all species. Finally, nutrient starvation (N−) had a small but significant effect on the ash (dry inorganic) content of macroalgae (ANOVA, $F_{1,36} = 28.5$, $P < 0.05$), and in contrast to the other effects, this was not species-dependent, there being no interaction between species and the starvation treatment (ANOVA, $P = 0.11$).

There was a significant effect of the washing treatment on the ash content of all macroalgae (ANOVA, $F_{1,36} = 425.8$, $P < 0.05$) as predicted, with the largest effect on marine species (Fig. 4). Washing had the most significant effect on *Derbesia* (A−), reducing the ash content by 83 ± 2% on average (±1SE) after three washing cycles. This was followed by *Ulva* (A−) with a reduction in the ash content of 43 ± 3% on average after three washing cycles. For freshwater *Oedogonium*, which initially had a low ash content, the washing treatment was less effective but still reduced the ash content by 7 ± 2%. These changes in ash content related to changes in specific elements, including key metals and halides in the marine species. For example, sodium decreased markedly by 98 ± 0% for *Derbesia* (A−) and 94 ± 1% for *Ulva* (A−), while chlorine decreased by 99 ± 0% for *Derbesia* (A−) and 90 ± 2% for *Ulva* (A−). Similarly, washing significantly reduced potassium and magnesium in *Derbesia* (A−) by 97 ± 0% and 69 ± 2% respectively. Potassium was reduced by 65 ± 6% in *Ulva* (A−). Consequently, the washing treatment led to a concomitant increase in the organic content of marine macroalgae. The content of carbon and therefore HHV of washed biomass increased by 29 ± 2% for *Derbesia* (A−) and 12 ± 2% for *Ulva* (A−).

Of the three species investigated, freshwater *Oedogonium* generally had the highest content of carbon and energy, whereas *Derbesia* had the highest content of protein and lipid. The combination of starvation and washing (N−/A−) was effective in producing biomass with a low protein content, reaching a minimum of 4.7% for *Oedogonium*, 4.8% for *Ulva* and 7.2% for *Derbesia*, corresponding to the lowest nitrogen content of 1.0% for *Oedogonium*, 1.1% for *Ulva* and 1.5% for *Derbesia* (Table 1). This combination of treatments (N−/A−) also produced the biomass with the lowest ash content of 3.4% for *Derbesia*, 6.1% for *Oedogonium* and 14.9% for *Ulva*.

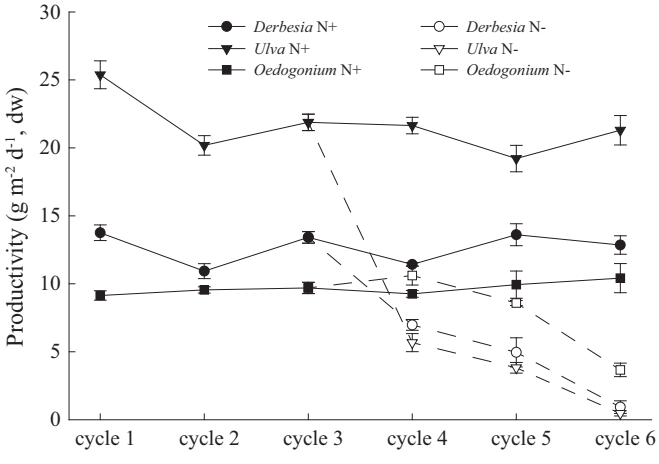

Fig. 2. Biomass productivity of macroalgae. Data show biomass productivity (average of samples, n = 4, ±1SE) on dry weight basis, of not starved (N+) and starved (N−) macroalgae over 6 culture cycles of 6 days. The nutrient starvation treatment starts after cycle 3.

3.3. HTL product yield

Fig. 5 shows the effect of the starvation and washing treatments on the yield of biocrude produced during the HTL processing of the three macroalgae species, on an ash-free dry weight basis (afdw). Of the two treatments, only nutrient starvation of the biomass had a significant effect on the yield of biocrude for *Derbesia* (ANOVA, $F_{1,12} = 20.4$, $P < 0.05$) and *Oedogonium* (ANOVA, $F_{1,12} = 9.3$, $P < 0.05$). Washing increased the yield of biocrude on a dry weight basis, but this was only the result of processing biomass with higher organic content, which compensated for the loss of inorganic material through washing. On an ash-free dry weight basis, washing had no significant effect on the yield of biocrude (ANOVA $F_{1,36} = 0.7$, $P = 0.42$).

When not starved of nutrients (N+), *Derbesia* had the highest yield of biocrude in the range of 38.6 − 41.7% afdw, compared to a yield in the range of 35.6 − 38.8% afdw for *Oedogonium* and 32.3 − 32.6% afdw for *Ulva*. The starved biomass (N−) that was inherently lower in

Table 1
Proximate, biochemical, ultimate and elemental analyses of macroalgae.

Species	*Derbesia*				*Ulva*				*Oedogonium*			
Treatments	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−
Proximate (wt.%)												
Ash	27.4	5.0	22.8	3.4	27.6	15.4	26.0	14.9	7.0	6.7	6.7	6.1
Moisture	8.8	8.0	8.7	8.3	11.9	9.5	12.2	10.6	7.0	7.2	6.9	7.8
Biochemical (wt.%)												
Lipid	11.1	11.2	4.9	5.5	2.1	2.1	1.2	1.4	7.4	8.5	3.1	3.2
Protein	25.0	33.5	7.9	7.2	18.2	21.0	4.8	4.8	19.7	19.8	6.7	4.7
Carbohydrate	27.7	42.4	55.8	75.5	40.1	52.0	55.8	68.3	58.9	57.8	76.6	78.2
Ultimate (wt.%)												
C	36.1	48.0	34.0	42.9	31.1	35.9	30.5	33.1	44.1	44.4	41.2	41.0
H	5.8	7.3	5.7	6.8	5.5	6.1	5.5	5.9	6.7	6.7	6.4	6.4
O	29.4	33.3	38.9	45.5	42.1	45.1	49.2	53.3	38.8	39.2	46.6	46.9
N	5.2	7.0	1.6	1.5	4.0	4.6	1.0	1.1	4.2	4.2	1.4	1.0
S	1.9	1.0	1.6	0.4	4.9	4.7	5.2	5.4	0.2	0.2	0.2	0.1
HHV[a]	16.5	21.9	14.7	18.2	13.5	15.5	12.6	13.6	19.2	19.3	17.1	17.0
C:N	6.9	6.9	20.7	28.5	7.9	7.9	29.4	31.5	10.5	10.5	28.8	40.6
Elemental (g/kg)												
Cl	95.9	0.5	81.9	0.6	56.1	8.0	35.2	1.6	3.2	3.5	4.5	4.7
Na	55.8	0.8	49.0	0.8	28.3	0.7	27.6	3.0	3.3	3.1	0.7	0.8
K	19.0	0.6	16.0	0.6	24.6	4.6	17.9	8.1	10.8	12.7	19.1	18.8
Mg	12.4	4.5	8.5	2.2	33.8	33.6	29.3	32.3	3.2	3.2	3.2	3.1
Ca	5.7	6.2	5.4	6.8	4.1	8.6	4.7	11.4	3.9	4.4	3.0	3.1
Fe	0.8	1.2	0.3	0.4	0.5	0.6	0.2	0.2	1.0	1.0	0.4	0.4
P	4.2	3.0	1.3	0.9	2.4	2.3	0.7	0.7	3.1	3.6	0.6	0.6

Data show biomass properties (average of samples, n = 4, dry weight basis) of macroalgae not starved (N+), starved (N−), not washed (A+) and washed (A−). Carbohydrate is determined by difference.
[a] HHV (MJ/kg) = higher heating value.

carbon and energy, generally yielded less biocrude than the biomass that was not starved (N+). However, the response to starvation ultimately varied among species with an interaction effect between species and the starvation treatment (ANOVA, $F_{1,36} = 5.1$, $P < 0.05$). The reduction was highest for *Derbesia*, where the yield decreased by 19 ± 3% on average (±1SE), compared to *Oedogonium* and *Ulva*, where the yields decreased by 13 ± 4% and 0 ± 6% respectively. These reductions led to yields in the range of 31.4 − 33.4% afdw for starved *Derbesia*, 32.2 − 32.6% afdw for starved *Oedogonium* and 30.6 − 34.0% afdw for starved *Ulva*.

Fig. 6 shows that the yield of biochar varied from 4% to 20% on an ash-free dry weight basis across all species and treatments. The starvation of biomass (N−) led to a decreased yield of biochar by 37 ± 7% on average across species, compared with biomass that was not starved (N+) (ANOVA, $F_{1,36} = 32.5$, $P < 0.05$). This decrease was largest for *Ulva* (50 ± 4%) and *Oedogonium* (43 ± 5%). In contrast, the post-harvest washing of biomass had no significant effect on the yield of biochar (ANOVA, $F_{1,36} = 0.0$, $P = 0.88$).

3.4. HTL product characterization

As hypothesized, the quality of biocrude was improved by the starvation treatment, which was manifested through important changes in the key quality parameters of nitrogen and sulfur contents, as shown in Table 2. Starved biomass (N−) produced a biocrude that was lower in nitrogen compared to biomass that was not starved (N+), with an average (±1SE) decrease in the content of nitrogen in

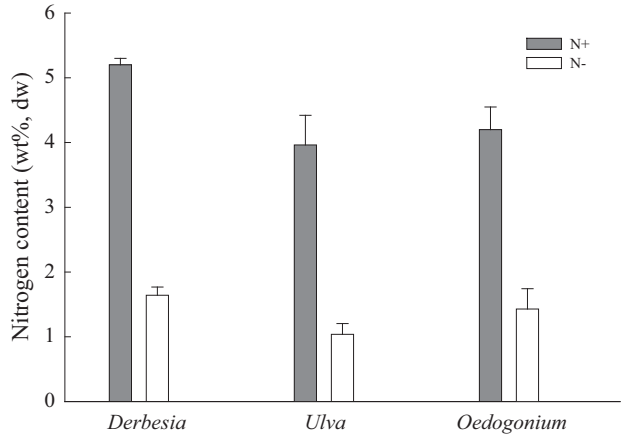

Fig. 3. Effect of starvation treatment on macroalgae nitrogen content. Data show biomass nitrogen content (average of samples, n = 4, ±1SE) on dry weight basis, of not starved (N+) and starved (N−) macroalgae.

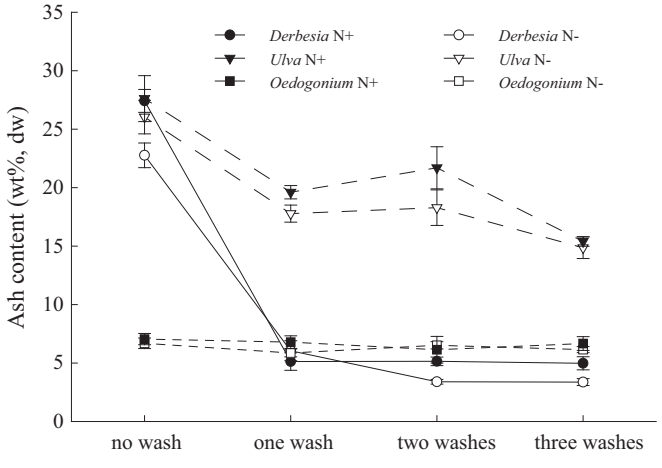

Fig. 4. Effect of washing treatment on macroalgae ash content. Data show biomass ash content (average of samples, n = 4, ±1SE) on dry weight basis, of not starved (N+) and starved (N−) macroalgae, washed three times with freshwater.

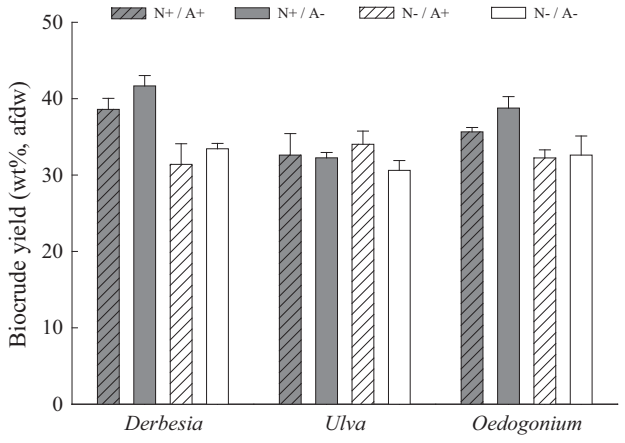

Fig. 5. Effect of starvation and washing treatments on biocrude yield. Data show biocrude yield (average of samples, n = 4, ±1SE) on an ash-free dry weight basis, following HTL of macroalgae not starved (N+), starved (N−), not washed (A+) and washed (A−).

the biocrude of 51 ± 1% for *Derbesia*, 53 ± 2% for *Ulva*, and 59 ± 0% for *Oedogonium*. Similarly, the content of sulfur in the biocrude decreased markedly by 66 ± 2% for starved *Derbesia*, 64 ± 4% for starved *Ulva* and 88 ± 0% for starved *Oedogonium*. In contrast, nutrient starvation had no effect on the content of carbon and hydrogen in the biocrude, with consistent values ranging from 72–74% for carbon, 7–8% for hydrogen and 31–34 MJ kg^{-1} for the HHV across all species and treatments. The decrease of the nitrogen and sulfur contents of biocrude, as a result of starvation, was consequently compensated for by an increase in the oxygen content to absolute values ranging from 14.8% to 17.2% for all starved biomass. The washing of biomass had no effect on the elemental composition of biocrude.

Of the three species, starved *Oedogonium* produced the biocrude with the lowest nitrogen contents of 2.1% and 2.2%, whether washed or not (N−/A− and N−/A+), followed by *Ulva* at 3.0% (N−/A−) and 2.7% (N−/A+) and *Derbesia* at 3.0% (N−/A−) and 3.2% (N−/A+). These biocrudes were also the lowest in sulfur, with concentrations at the ppm level for *Oedogonium* (below the limit of detection) and ranging between 0.2 and 0.3% for *Derbesia* and *Ulva*.

The quality of biochar produced by the HTL of macroalgae was also only influenced by the starvation treatment (Table 2). The biochars produced from starved biomass (N−) generally had a higher content of carbon and hydrogen than biochars from the untreated biomass (N+), with higher energy values ranging between 18 and 20 MJ/kg for starved *Derbesia*, 13–16 MJ/kg for starved *Ulva* and 25 MJ/kg for starved *Oedogonium*. Interestingly, the biochar produced from starved *Oedogonium* contained up to 60% carbon with a relatively low inorganic content in the range of 26–28% (calculated by subtraction of the sum of C, H, O and N percentages from 100%).

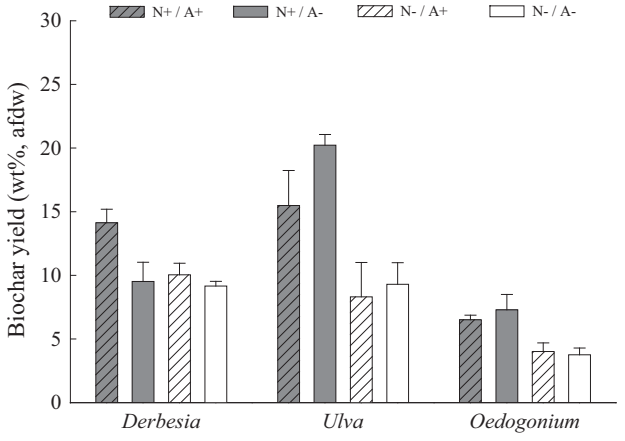

Fig. 6. Effect of starvation and washing treatments on biochar yield. Data show biochar yield (average of samples, n = 4, ±1SE) on an ash-free dry weight basis, following HTL of macroalgae not starved (N+), starved (N−), not washed (A+) and washed (A−).

The effect of treatments on the composition of the aqueous phase was assessed by measuring the concentration of total organic (TOC) and inorganic carbon (TIC) and total nitrogen (TN). For all three species, the aqueous phase produced from the starved biomass (N−) had a concentration of TOC that was reduced by 17 ± 6% compared to biomass that was not starved (N+). There were similar reductions in TIC of 86 ± 9%, and TN of 74 ± 1% (Table 2). The concentration of TOC ranged from 3807 mg/L for starved biomass to 6921 mg/L for biomass that was not starved, and was noticeably higher than the concentration of TIC at ≤650 mg/L across all species and treatments. The concentration of TN in the aqueous phase was relatively consistent across all species, and was lower for starved biomass (272–465 mg/L) that had initially less nitrogen, compared with biomass that was not starved (1021–1636 mg/L).

3.5. Chemical energy recovery and mass balance

While the heteroelements nitrogen and sulfur decreased with the various treatments, it is instructive to examine how these lower values partition across the product streams. Thus, the elemental and energy recoveries of the HTL products presented in Table 3 were determined from the ultimate analysis of biocrude and biochar, and calculated by difference for the remaining combined aqueous and gas products. Importantly, the two treatments of nutrient starvation and washing had a substantial effect on the distribution of nitrogen and sulfur. The mass balance shows that most of the nitrogen did not report to the char, but was partitioned between the biocrude and the combined aqueous and gas phases. The relative recovery of nitrogen in the biocrude increased with the treatments, following the trend: untreated < washed < starved < starved and washed biomass. In terms of nitrogen partitioning, HTL of starved and washed biomass (N−/A−) had the effect of pushing a higher proportion of the biomass nitrogen into the biocrude fraction. This differed from untreated algal samples (N+/A+), in which the combined aqueous and gas phases ended up with proportionally more nitrogen than the biocrude. For example, N−/A− *Ulva* retained 64.4% of its nitrogen in the biocrude fraction, whereas the biocrude generated from N+/A+ *Ulva* only retained 31.8% of the biomass nitrogen.

The effect of both treatments on the sulfur content of macroalgae and consequently its distribution in HTL product streams was variable (Table 3). The starvation treatment (N−) led to a 65 ± 6% reduction in the recovery of sulfur in biocrude on average across all species, while the washing treatment had the opposite effect with a slight increase of the recovery of sulfur in the biocrude. For marine species and particularly for *Ulva*, the majority of the sulfur (76–99%) contained in the biomass was effectively excluded from the biocrude phase. For freshwater *Oedogonium* that has inherently low sulfur content, the same effect occurred with most of the sulfur excluded from the biocrude phase, particularly after starvation (81–92%), resulting in biocrudes with sulfur content at the ppm level. In a similar way, most of the oxygen did not report to the biocrude but to the combined aqueous and gas phases (87–94%), with a relatively high consistency in the distribution of oxygen in HTL product streams across treatments. As a result, the starved biomass (N−) that initially had a higher content of oxygen produced a biocrude that was also higher in oxygen, compared with biomass that was not starved (N+).

Despite a high variability in the elemental composition of the macroalgal feedstocks, there was little variation in the recovery of carbon and hydrogen in the biocrude (Table 3). This manifested through a slight decrease in the recovery of both elements after the starvation treatment (<5%, compared with untreated biomass), and a slight increase in their recovery following the washing treatment (<6%,

Table 2
Ultimate analysis of biocrude, biochar and aqueous products.

Species	*Derbesia*				*Ulva*				*Oedogonium*			
Treatments	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−
Biocrude (wt.%)												
C	71.9	72.2	73.5	73.5	71.9	73.2	73.1	72.0	71.7	71.7	71.7	72.3
H	7.7	7.9	7.6	7.5	7.2	8.1	6.9	6.9	7.4	7.3	6.6	7.3
O	11.7	11.3	14.8	14.8	12.0	11.9	16.2	15.9	13.8	13.8	17.2	17.0
N	6.1	6.7	3.2	3.0	6.4	5.7	2.7	3.0	5.3	5.3	2.2	2.1
S	0.8	0.7	0.3	0.2	0.7	0.5	0.2	0.2	0.4	0.3	0.0	0.0
HHV[a]	33.0	33.3	33.0	32.9	32.3	33.8	32.0	31.6	32.2	32.2	31.1	32.1
Biochar (wt.%)												
C	28.1	29.7	40.1	45.6	16.3	8.0	34.5	28.5	38.9	41.5	60.0	59.7
H	3.5	2.9	3.0	3.3	2.0	1.6	2.7	2.1	3.2	3.3	4.3	4.2
O	2.7	2.8	3.2	3.7	2.8	1.8	4.0	3.2	4.2	4.3	6.0	4.6
N	2.0	3.1	2.4	2.7	1.4	0.7	1.6	1.3	3.6	3.9	2.6	2.7
S	7.0	5.6	6.6	4.3	14.9	18.1	10.4	13.0	0.6	0.9	1.1	0.7
HHV[a]	14.3	14.0	17.9	19.9	9.3	6.3	15.8	13.3	16.9	18.0	25.5	25.3
Aqueous (mg/L)												
TOC	5750	6214	3807	5150	4750	5343	4064	4071	6921	5843	6121	5743
TIC	557	650	14	29	414	414	29	29	93	100	21	43
TN	1636	1421	465	398	1243	1286	317	272	1043	1021	272	274

Data show ultimate analysis (average of samples, n = 4, dry weight basis) of HTL products following conversion of macroalgae not starved (N+), starved (N−), not washed (A+) and washed (A−).
[a] HHV (MJ/kg) = higher heating value; TOC = total organic carbon; TIC = total inorganic carbon; TN = total nitrogen.

compared with untreated biomass). This relatively consistent recovery of carbon in biocrude across treatments was best illustrated by a plot of biomass carbon content and biocrude yield in Fig. 7, which showed that the two variables were strongly correlated ($R^2 = 0.96$). The line shown in this figure represents a linear correlation of the data, within a carbon content of biomass in the range of 29.7–48.2%:

$$Y_{BIOCRUDE} = 0.885 \times C_{BIOMASS} - 7.455 \quad (3)$$

, where $Y_{BIOCRUDE}$ is the yield (dw) of biocrude and $C_{BIOMASS}$ is the carbon content (dw) of the biomass. *Ulva* and *Oedogonium* had a relatively narrow range of carbon values across treatments, however, the biocrude yield varied linearly across a wide range of biomass carbon values for *Derbesia*.

Approximately half of the biomass energy was transferred to the biocrude with most of the remainder transferred to the combined aqueous and gas phases.

4. Discussion

The results demonstrate that it is indeed possible to effectively manipulate the composition of macroalgal biomass through pre- and post-harvest treatments. The nutrient starvation of the cultures and washing of the biomass, individually or combined, significantly affected the quality of macroalgal feedstocks. Restricting the supply of nutrients to macroalgal cultures for 18 days resulted in an effective reduction of the content of nitrogen and sulfur in biomass, and consequently an effective reduction in the content of nitrogen and sulfur in biocrude.

Table 3
Element conversion ratio and energy recovery in HTL product streams.

Species		*Derbesia*				*Ulva*				*Oedogonium*			
Treatments		N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−
C	Biocrude	49.0	54.5	46.6	50.6	45.1	49.3	50.1	49.6	49.8	53.9	48.5	49.5
	Biochar	7.0	5.1	8.1	8.6	4.9	3.4	5.7	5.9	4.9	5.9	5.1	4.7
	Aq. + Gas	44.0	40.4	45.3	40.8	50.0	47.3	44.2	44.5	45.2	40.3	46.4	45.7
H	Biocrude	32.7	39.5	28.6	32.7	25.5	32.0	26.4	26.6	33.7	36.3	29.0	32.3
	Biochar	5.4	3.3	3.7	3.9	3.4	3.9	2.4	2.4	2.7	3.1	2.3	2.1
	Aq. + Gas	61.9	57.1	67.8	63.4	71.1	64.1	71.2	71.0	63.7	60.6	68.7	65.6
O	Biocrude	9.7	12.3	8.2	9.6	5.6	6.4	6.9	6.8	10.9	11.7	10.3	10.2
	Biochar	0.8	0.7	0.6	0.7	0.6	0.6	0.4	0.4	0.6	0.7	0.4	0.3
	Aq. + Gas	89.4	87.0	91.3	89.7	93.8	93.0	92.7	92.8	88.5	87.6	89.3	89.5
N	Biocrude	28.8	34.7	41.8	58.7	31.8	30.0	55.1	64.4	38.7	42.2	43.5	58.8
	Biochar	3.5	3.7	9.8	14.4	3.3	2.3	7.7	8.3	4.9	5.8	6.3	8.6
	Aq. + Gas	67.7	61.6	48.4	26.9	64.9	67.7	37.2	27.3	56.5	52.0	50.2	32.6
S	Biocrude	10.3	23.8	4.3	12.2	2.8	2.5	0.7	1.0	58.2	50.7	8.0	19.1
	Biochar	33.3	46.4	29.1	77.5	28.2	57.9	10.2	16.5	16.2	27.7	24.2	40.8
	Aq. + Gas	56.4	29.8	66.6	10.3	69.0	39.6	89.1	82.5	25.6	21.6	67.7	40.2
ER	Biocrude	49.1	55.3	48.5	53.2	46.9	52.8	53.3	53.1	51.4	55.6	50.6	53.1
	Biochar	7.8	5.3	8.4	8.8	6.4	6.1	6.4	6.8	4.9	5.8	5.2	4.8
	Aq. + Gas	43.0	39.4	43.2	37.9	46.7	41.1	40.3	40.1	43.7	38.6	44.2	42.0

Data show element conversion ratio and energy recovery (wt.%, dry weight basis) in HTL products following conversion of macroalgae not starved (N+), starved (N−), not washed (A+) and washed (A−). Aqueous and gas products are combined and determined by difference (Aq. + Gas).

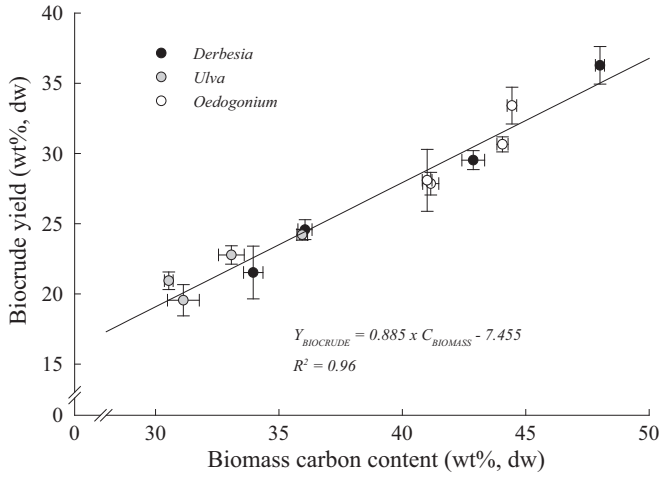

Fig. 7. Effect of biomass carbon content on biocrude yield. Data show the effect of biomass carbon content on biocrude yield (dry weight) for the three species of macroalgae (average of samples, n = 4 per treatment, ±1SE).

However, starved biomass was also lower in carbon per unit of mass and this led to a decreased yield of biocrude. The decrease in carbon was due to the modification of the organic profile of the biomass, with a lower proportion of proteins and lipids, and a higher proportion of carbohydrates that are less dense in carbon. Most importantly, starvation resulted in a major decrease in biomass productivities that is due to the essential metabolic roles of nutrients, particularly nitrogen, in photosynthesis, the synthesis of proteins, and the catalytic capacity of enzymes [26]. Therefore, the efficiency of starvation in enhancing the quality of biocrude is offset by a simultaneous decrease of the overall yield and productivity of biocrude, as highlighted in Table 4. Furthermore, the efficiency of starvation in reducing the content of nitrogen and sulfur in biocrude is balanced by an increase of the oxygen content of the biocrude, which counteracts some of the potential benefits made in terms of the requirements for the upgrading of biocrude to a blendable fuel.

Washing macroalgal biomass with freshwater to reduce the ash content (i.e. inorganic salts, alkali metals and halides) was globally beneficial to the biocrude production process. For all species, washing was effective in removing the external salts (trapped between algae blades or filaments) following harvesting and dewatering. In addition, we believe that a second mechanism of osmosis caused a variation in the response of the species to the treatment. The exceptional decrease of ash content in *Derbesia* (>80%) was most likely due to the coenocytic siphonous structure of the alga, composed of a single giant cell [27], enabling direct contact and passive diffusion of osmolytes between the entire internal cytoplasm and the external medium (freshwater). The osmotic effect was less effective at removing the internal salts of *Ulva* which is two cells thick, or of the freshwater *Oedogonium* that already has a low internal ash content. Washing did not affect biocrude production, where the loss of (inorganic) biomass harvested per unit area was compensated for by higher biocrude yields per unit of biomass processed (on a dry basis). Importantly, the major benefit of using washed biomass, especially for processing of marine species, will be a reduced load of ash in the continuous flow reactor reducing the effects of corrosion [7,17]. It is also important to consider the effect of washing in terms of life cycle analysis [28], where the organic fraction is concentrated within the biomass (higher energy content), therefore increasing the efficiency of biomass transportation and processing. The disposal of the water used to wash the biomass, containing dissolved inorganic salts (1–5 g/L, when using 3 L/100 g of algae), could be achieved through its recycling in marine macroalgal cultures to compensate for evaporation.

In this study, the whole nutrient supply to the cultures (not only nitrogen) was restricted to evaluate the effect of the starvation treatment in a scaled-up algal cultivation concept. In this concept, wastewater is used as a cost-effective nutrient source to grow algae at optimal conditions [29], before transferring the biomass to a nutrient-free environment (polishing tank) in order to reduce the nitrogen content. Of the species of macroalgae investigated, the freshwater *Oedogonium* showed promising results, with the ability to be starved of nutrient for a period of 6–8 days without a significant impact on productivity. After an extended period of starvation of 18 days, the liquefaction of starved *Oedogonium* produced a biocrude of high quality with a low content of nitrogen (2%) and sulfur (<0.1%), reducing the hydrogen requirement for biocrude upgrading into a blendable fuel. The nitrogen content of the biocrudes reported in this study for starved macroalgae, particularly *Oedogonium*, is noticeably lower than the nitrogen content of biocrudes reported to date in other HTL studies of algae (listed in Frank et al. [10]), which demonstrates the potential benefits of the starvation treatment. For other algal species, and especially for marine species for which nutrient starvation had a major impact on biomass productivity, the assessment of profitability in terms of biocrude productivity and refining costs (hydrogen demand for the hydrotreatment) will determine if the starvation treatment of the cultures is beneficial. For this assessment, it will be critical to determine

Table 4
Summary table of the productivity and yield of biomass, biocrude and biochar.

Species	*Derbesia*				*Ulva*				*Oedogonium*			
Treatments	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−	N+ A+	N+ A−	N− A+	N− A−
Biomass[a]												
Productivity (g/m^2/d dw)	12.6	9.3	4.3	3.3	20.7	16.7	3.3	2.7	9.9	9.8	7.6	7.6
Productivity (g/m^2/d afdw)	8.1	8.1	2.9	2.9	12.5	12.5	2.0	2.0	8.5	8.5	6.6	6.6
Biocrude												
Yield (% afdw)	38.6	41.7	31.4	33.4	32.6	32.3	34.0	30.6	35.6	38.8	32.2	32.6
Productivity (g/m^2/d afdw)	3.1	3.4	0.9	1.0	4.1	4.0	0.7	0.6	3.0	3.3	2.1	2.1
Heteroatoms (wt.%)												
O	11.7	11.3	14.8	14.8	12.0	11.9	16.2	15.9	13.8	13.8	17.2	17.0
N	6.1	6.7	3.2	3.0	6.4	5.7	2.7	3.0	5.3	5.3	2.2	2.1
S	0.8	0.7	0.3	0.2	0.7	0.5	0.2	0.2	0.4	0.3	0.0	0.0
Biochar												
Yield (% afdw)	14.1	9.5	10.0	9.2	15.5	20.2	8.3	9.3	6.5	7.3	4.0	3.8
Productivity (g/m^2/d afdw)	1.1	0.8	0.3	0.3	1.9	2.5	0.2	0.2	0.6	0.6	0.3	0.2

[a] Values for biomass productivity are an average of the three last weeks of culture (starvation phase).

the length and intensity (nutrient concentration) of the starvation treatment, if at all, on a species by species basis.

Furthermore, the relationship between the nitrogen content of biomass and biocrude showed that a higher proportion of biomass nitrogen was recovered in the biocrude phase for (starved) biomass that contained a lower proportion of nitrogen. Given that nutrient starvation could be detrimental to the aim of recycling nitrogen and the overall productivity of biocrude production, the possibilities of selectively extracting nitrogen from biomass as protein, prior to HTL of the residual biomass, is a key area of future research.

In terms of conversion efficiency, *Oedogonium* showed promising results with a high yield of biocrude (36–39% afdw), even after starvation (32–33% afdw). It is also important to note that *Oedogonium* had the lowest productivity of the three species investigated, however, higher productivities of 15–20 g/m^2/d dw at large-scale have been demonstrated by Cole et al. [30]. *Derbesia* that was not starved of nutrients afforded the highest yield of biocrude (39–42% afdw) of the three species, and this was higher than the yield previously reported [4]. It was also higher than yields reported in the literature for green and brown seaweeds [9,31]. These higher yields are most likely due to a lower ash content and a higher lipid content than the marine species that have been the focus of research to date [9,31], and therefore a higher proportion of organic carbon. Furthermore, these yields are comparable to the middle range of yields obtained for several microalgae and cyanobacteria species processed under similar conditions, including *Dunaliella tertiolecta*, *Chlorella vulgaris* and *Spirulina platensis* [10,32–34] The data presented here confirm that yields of >35% afdw biocrude can be achieved through the HTL of low-lipid feedstocks such as micro- [35] and macroalgae.

Notably, *Ulva* had the lowest biocrude yield of all three species, mainly due to a low carbon content, and this yield was not affected by the starvation treatment for the same reason. However, *Ulva* had the highest productivity of the three species, and consequently the highest productivity of biocrude in untreated conditions (4.1 g biocrude/m^2/d dw, compared to 3.0–3.1 g biocrude/m^2/d afdw for *Derbesia* and *Oedogonium*), highlighting that selecting species with a high biocrude yield is not systematically the preferred option (Table 4), unless a high biomass productivity can also be achieved [3,36]. The optimisation of biomass productivities is therefore central to improving efficiencies in the production of biocrude. Similarly, several studies showed that the operating conditions of HTL including temperature, solid loading, residence time, and the use of heterogeneous catalysts greatly influence biocrude yield and composition, and the optimisation of the operating parameters will also be critical in achieving maximum recovery of biomass energy [34,36,37].

Finally, a significant portion of biomass energy was also recovered in the biochar, aqueous and gaseous co-products. The starvation of biomass resulted in a lower yield and higher quality of biochar, with higher carbon and energy and a lower inorganic fraction than biochar produced from biomass that was not starved. This high carbon and low ash biochar is suitable for agriculture as a soil ameliorant and fertilizer and could add value to the overall production process while providing benefits for long term sequestration of carbon [38]. In contrast to biochar, a high portion of the biomass energy was recovered in the combined aqueous and gas phases. The aqueous phase has been the focus of studies investigating the recycling of nutrients (N, P, K) back into algal cultures [14,39–41] or the gasification of the organics to recover some energy as hydrogen [42], to add value to the overall process. Similarly, the carbon dioxide that forms most of the gas phase could be recycled back into algal cultures to enhance growth [43,44]. The recovery of all co-products from HTL will be critical to increase the value of the algal biocrude production process [43].

5. Conclusion

In conclusion, this study has demonstrated that macroalgae can be manipulated in culture, and in post-harvest processing, to specifically improve the composition of feedstock for the production of biocrude. The treatments of nutrient starvation and the washing of biomass were effective in reducing the content of nitrogen, sulfur and ash in biomass, which resulted in an improved quality of biocrude. While further optimisation of the HTL process will improve the recovery of biomass energy to biocrude, we demonstrate that the optimisation of culture protocols and post-harvest processing is a powerful tool to add viability to the algae-to-biofuel concept.

Acknowledgments

This project is supported by the Australian Government through the Australian Renewable Energy Agency (ARENA, 002369), the Australian Research Council (DP1096802), the Science and Industry Endowment Fund (RP03-028) and the Advanced Manufacturing Cooperative Research Centre (AMCRC) funded through the Australian Government's Cooperative Research Centre Scheme. This research is part of the MBD Energy Research and Development program for Biological Carbon Capture and Storage (Bio CCS).

References

[1] A.B. Ross, J. Jones, M. Kubacki, T. Bridgeman, Classification of macroalgae as fuel and its thermochemical behaviour, Bioresour. Technol. 99 (2008) 6494–6504.
[2] J. Rowbotham, P. Dyer, H. Greenwell, M. Theodorou, Thermochemical processing of macroalgae: a late bloomer in the development of third-generation biofuels? Biofuels 3 (2012) 441–461.
[3] N. Neveux, M. Magnusson, T. Maschmeyer, R. de Nys, N.A. Paul, Comparing the potential production and value of high-energy liquid fuels and protein from marine and freshwater macroalgae, Glob. Change Biol. Bioenergy (2014), http://dx.doi.org/10.1111/gcbb.12171.
[4] N. Neveux, A.K.L. Yuen, C. Jazrawi, et al., Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae, Bioresour. Technol. 155 (2014) 334–341.
[5] S.S. Toor, L. Rosendahl, A. Rudolf, Hydrothermal liquefaction of biomass: a review of subcritical water technologies, Energy 36 (2011) 2328–2342.
[6] P. Biller, A.B. Ross, Hydrothermal processing of algal biomass for the production of biofuels and chemicals, Biofuels 3 (2012) 603–623.
[7] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr., J.W. Tester, Thermochemical biofuel production in hydrothermal media: a review of sub-and supercritical water technologies, Energy Environ. Sci. 1 (2008) 32–65.
[8] D. López Barreiro, W. Prins, F. Ronsse, D.W. Brilman, Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects, Biomass Bioenergy 53 (2013) 113–127.
[9] D. Zhou, L. Zhang, S. Zhang, H. Fu, J. Chen, Hydrothermal liquefaction of macroalgae *Enteromorpha prolifera* to bio-oil, Energy Fuel 24 (2010) 4054–4061.
[10] E.D. Frank, A. Elgowainy, J. Han, Z. Wang, Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae, Mitig. Adapt. Strateg. Glob. Chang. 18 (2013) 137–158.
[11] Z. Li, P.E. Savage, Feedstocks for fuels and chemicals from algae: treatment of crude bio-oil over HZSM-5, Algal Res. 2 (2013) 154–163.
[12] P. Duan, P.E. Savage, Upgrading of crude algal bio-oil in supercritical water, Bioresour. Technol. 102 (2011) 1899–1906.
[13] P. Duan, P.E. Savage, Catalytic hydrotreatment of crude algal bio-oil in supercritical water, Appl. Catal. B Environ. 104 (2011) 136–143.
[14] D.C. Elliott, T.R. Hart, A.J. Schmidt, et al., Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor, Algal Res. 2 (2013) 445–454.
[15] P. Duan, P.E. Savage, Catalytic treatment of crude algal bio-oil in supercritical water: optimization studies, Energy Environ. Sci. 4 (2011) 1447–1456.
[16] S.G. Roussis, R. Cranford, N. Sytkovetskiy, Thermal treatment of crude algae oils prepared under hydrothermal extraction conditions, Energy Fuel 26 (2012) 5294–5299.
[17] C. Jazrawi, P. Biller, A.B. Ross, A. Montoya, T. Maschmeyer, B.S. Haynes, Pilot plant testing of continuous hydrothermal liquefaction of microalgae, Algal Res. 2 (2013) 268–277.
[18] M.N. Merzlyak, O.B. Chivkunova, O.A. Gorelova, I.V. Reshetnikova, A.E. Solovchenko, I. Khozin-Goldberg, Z. Cohen, Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta) 1, J. Phycol. 43 (2007) 833–843.
[19] A.R. Angell, L. Mata, R. de Nys, N.A. Paul, Variation in amino acid content and its relationship to nitrogen content and growth rate in *Ulva ohnoi* (Chlorophyta), J. Phycol. 50 (2014) 216–226.
[20] M. Giordano, A. Norici, S. Ratti, J.A. Raven, Role of sulfur for algae: acquisition, metabolism, ecology and evolution, in: R. Hell, C. Dahl, D.B. Knaff, T. Leustek (Eds.), Sulfur Metabolism in Phototrophic Organisms, Springer, Dordrecht, ISBN: 978-1-4020-6862-1, 2008, pp. 397–408.
[21] M. Magnusson, L. Mata, R. de Nys, N.A. Paul, Biomass, lipid and fatty acid production in large-scale cultures of the marine macroalga *Derbesia tenuissima* (Chlorophyta), Mar. Biotechnol. 16 (2014) 456–464.

[22] R.J. Lawton, L. Mata, R. de Nys, N.A. Paul, Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: selecting target species and strains, PLoS ONE 8 (2013) e77344.
[23] R.J. Lawton, R. de Nys, N.A. Paul, Selecting reliable and robust freshwater macroalgae for biomass applications, PLoS ONE 8 (2013) e64168.
[24] R.R.L. Guillard, J.H. Ryther, Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve, Can. J. Microbiol. 8 (1962) 229–239.
[25] S.A. Channiwala, P.P. Parikh, A unified correlation for estimating HHV of solid, liquid and gaseous fuels, Fuel 81 (2002) 1051–1063.
[26] M. Hein, M.F. Pedersen, K. Sand-Jensen, Size-dependent nitrogen uptake in micro- and macroalgae, Mar. Ecol. Prog. Ser. 118 (1995) 247–253.
[27] C.S. Lobban, P.J. Harrison, Morphology, life histories, and morphogenesis, Seaweed Ecology and Physiology, Cambridge University press, Cambridge, ISBN: 9780521408974, 1996, pp. 17–35.
[28] S. Grierson, V. Strezov, J. Bengtsson, Life cycle assessment of a microalgae biomass cultivation, bio-oil extraction and pyrolysis processing regime, Algal Res. 2 (2013) 299–311.
[29] G.W. Roberts, M.-O.P. Fortier, B.S. Sturm, S.M. Stagg-Williams, Promising pathway for algal biofuels through wastewater cultivation and hydrothermal conversion, Energy Fuel 27 (2013) 857–867.
[30] A.J. Cole, R. de Nys, N.A. Paul, Removing constraints on the biomass production of freshwater macroalgae by manipulating water exchange to manage nutrient flux, PLoS ONE 9 (2014) e101284.
[31] K. Anastasakis, A.B. Ross, Hydrothermal liquefaction of the brown macro-alga *Laminaria saccharina*: effect of reaction conditions on product distribution and composition, Bioresour. Technol. 102 (2011) 4876–4883.
[32] T. Minowa, S.-Y. Yokoyama, M. Kishimoto, T. Okakura, Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction, Fuel 74 (1995) 1735–1738.
[33] U. Jena, K. Das, J. Kastner, Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*, Bioresour. Technol. 102 (2011) 6221–6229.
[34] P. Biller, A.B. Ross, Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content, Bioresour. Technol. 102 (2011) 215–225.
[35] G. Yu, Y. Zhang, L. Schideman, T. Funk, Z. Wang, Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil, Trans. ASABE 54 (2011) 239–246.
[36] L. Garcia Alba, C. Torri, C. Samorì, J. Van Der Spek, D. Fabbri, S.R. Kersten, D.W. Brilman, Hydrothermal treatment (HTT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept, Energy Fuel 26 (2012) 642–657.
[37] C. Torri, L. Garcia Alba, C. Samorì, D. Fabbri, D.W. Brilman, Hydrothermal treatment (HTT) of microalgae: detailed molecular characterization of HTT oil in view of HTT mechanism elucidation, Energy Fuel 26 (2012) 658–671.
[38] M.I. Bird, C.M. Wurster, P.H. de Paula Silva, N.A. Paul, R. de Nys, Algal biochar: effects and applications, Glob. Change Biol. Bioenergy 4 (2012) 61–69.
[39] 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.
[40] L. Garcia Alba, C. Torri, D. Fabbri, S.R.A. Kersten, D.W. Brilman, Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae, Chem. Eng. J. 228 (2013) 214–223.
[41] M. Pham, L. Schideman, J. Scott, N. Rajagopalan, M.J. Plewa, Chemical and biological characterization of wastewater generated from hydrothermal liquefaction of *Spirulina*, Environ. Sci Technol. 47 (2013) 2131–2138.
[42] D.C. Elliott, T.R. Hart, G.G. Neuenschwander, L.J. Rotness, G. Roesijadi, A.H. Zacher, J.K. Magnuson, Hydrothermal processing of macroalgal feedstocks in continuous-flow reactors, ACS Sustain. Chem. Eng. 2 (2013) 207–215.
[43] G. Yu, Y. Zhang, L. Schideman, T. Funk, Z. Wang, Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae, Energy Environ. Sci. 4 (2011) 4587–4595.
[44] A.J. Cole, L. Mata, N.A. Paul, R. de Nys, Using CO_2 to enhance carbon capture and biomass applications of freshwater macroalgae, Glob. Change Biol. Bioenergy (2013), http://dx.doi.org/10.1111/gcbb.12097.