HYDROTHERMAL EXTRACTION OF ALGAL FATTY ACID AND HYDROCHAR CHARACTERISATION.

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ABSTRACT

Several species of Microalgae are rich sources of lipids that are valuable feedstock for biodiesel production. However, there are significant technical and economic challenges to the development of algal biofuels, not least of which is the dehydration of the algal biomass before conventional lipid extraction. Hydrothermal carbonisation (HTC) of algal biomass is suitable for facile extraction of biodiesel-precursor triacylglycerides. During algae HTC, cell-rupture liberates lipids to the aqueous phase. These adsorb to the subsequently produced carbon-rich char and are removed from aqueous phase by filtration. Extraction of the lipids into an organic wash phase liberates lipids without prior drying of the algal biomass. This study aims to elaborate the quality of the extracted lipids for biofuels applications and the utility of the algal HTC char as a fuel and as a soil amendment. Algal biomass (*Phaeodactylum tricornutum*) was subjected to hydrothermal carbonisation at moderate conditions to yield a solid char from which lipids were extracted into hexane. Fatty acid methyl ester yield following subsequent transesterification was analysed qualitatively and quantitatively by gas chromatography-mass spectrometry to establish lipid recovery, yield and distribution. The solid char phase was subject to thermal gravimetric analysis to establish its fuel properties. The potential phytotoxicity of the solid product was assessed by a seed-germination bioassay.

Keywords: hydrothermal carbonization, algal biodiesel, biodiesel, biochar, hydrochar.

1. INTRODUCTION

Fossil fuels are in ultimately limited supply, taking millions of years to form (Khanal et al., 2010). The extensive use of such fossil fuels means their availability is decreasing and exhaustion expected within several decades (Fu *et al*., 2013), whilst the rapid oxidation of fossil carbon is increasing atmospheric carbon dioxide concentrations (IPCC 2013). The emergence of renewable, clean energy sources to replace fossil fuels is now being explored and biodiesel is aiding the transition from conventional fossil fuels to renewable liquid fuels for transport.

The use of microalgae as a feedstock to replace existing fossil fuels is becoming increasingly attractive due to environmental benefits and reduced food-biofuel competition (Hannon *et al*., 2010). Global biodiesel production from renewable sources has increased substantially, and concerns have been raised regarding the displacement of food crops for biofuels; increasing crops prices and allocated land for biomass cultivation (Singh, Nigam & Murphy, 2011). Due to photosynthetic processes of algae, burning algal biomass is carbon neutral. During photosynthesis, algae cells reproduce rapidly accumulating proteins, carbohydrates, nucleic acids and lipids, with the latter being of special interest in the subsequent production of biodiesel – biodiesel being a mixture of fatty acid methyl esters produced typically by the transesterification of biomass triacylglycerides. The algal proportion of lipids will vary depending on the culture conditions and algae strains (Demirbas, 2009).

The application of algae for commercial biodiesel production is promising. However, before the application of algae as biofuel becomes economically feasible, the demand

of energy per unit mass of algae harvested must be reduced (Lee and Shah, 2013). The predominant concern is that the energy required to dewater algae is large compared to the overall energy output from the triacylglycerides in the algae (Heilmann *et al*., 2011).

Hydrothermal carbonisation (HTC) of microalgae has received particular attention due to the potential to obtain algal oils without energy intensive removal of water (Heilmann *et al.*, 2011). HTC produces a hydrochar to which algal lipids are adsorbed and the process is thermodynamically favourable as hydrogen and oxygen are removed and carbon values are maintained (Valentas & Heilmann, 2011). Once filtered, hydrochar-bound lipids can be solvent extracted prior to transesterification.

Phaeodactylum tricornutum is a diatom with an oil content of 20 -30% (Khanal *et al*., 2010). Diatoms are important on aquatic ecosystems and have broad industrial potential (Huysman *et al*., 2010). *P. tricornutum* are able to acclimate to environmental changes, undergoing modification in their lipid metabolism. Substantial amounts of lipids in the form of triacylglycerides (TAG) can be stored under conditions such as photo oxidative stress and other unfavourable environmental changes (Hu et al., 2008). Polymerised silica within *P. tricornutum* cell wall supports the accumulation of TAG. Silica polymerisation requires lower energy compared with lignin-based cell walls contributing to energetic savings in carbon (Abbriano, 2012). Algal triacylglycerides are comprised of mid to long chain fatty acids bound to glycerol; transesterification by alcoholysis produces methyl esters of fatty acids which are the primary source for biodiesel (Gupta, 2011).

The present study aims to analyse quantitatively the fatty acid methyl ester yield available *via* hydrochar-mediated extraction of algal lipids from *P. tricornutum*. Furthermore, the co-produced hydrochar is of potential utility as a fuel and/or as a biochar: this study will investigate the fuel properties and potential phytotoxicity (an important limiting factor on the application of hydrochar as biochar) of the coproduced hydrochar.

2. METHODS

2.1 Hydrothermal carbonization of microalgae

The HTC reaction was run using a 100 ml Buchi MiniClave heated by a Julabo HE-4 oilbath. Four replicate preparations were done using 4 g samples of freeze-dried microalgae *Phaeodactylum tricornutum*. These were added to 50 ml of 0.1M citric acid solution and hydrothermally carbonised at approximately 210°C for 2 hours, under an average pressure of 20 bar. The four samples were filtered and washed with deionised water.

2.2 Delipidation of HTC char

To remove adsorbed lipids, HTC chars were stirred overnight in hexane (40 ml), gravity filtered and washed with hexane (40 ml approx.). The filter paper with the delipidated char was stored in the fridge and the filtrate evaporated by a water bath until only lipids remained.

2.3 Transesterification

Recovered lipids (25 mg) were added to a headspace vial and mixed with 0.5 M methanolic NaOH (1.25 ml). The process was repeated for triplicate C16, C18 and C20 fatty acid calibration standards. All the samples were heated at 100°C for 7 minutes, allowed to cool, mixed with 50% BF_3 (2 ml) and returned to the heat for 5 minutes. The samples were cooled to 30-40°C and mixed by shaking with hexane (2 ml) and saturated NaCl (5 ml) and left to separate. The top layer was collected to a headspace vial. Hexane (2 ml) was added a further two times to the samples and each time the top layer collected into the headspace vial. The samples collected were evaporated to dryness using nitrogen and hexane (2ml) added.

2.4 GC-MS analysis

Fatty acid methyl ester yields of transesterification were analysed by GC-MS as follows. Samples (1 µl) were injected at 300°C into a Perkin Elmer Elite 5 MS capillary column (30 m x 0.25 mm x 0.25um). The oven temperature was held at 60°C for 1 minute and raised to 300°C (20°C min^{-1}), where it was held for 7 minutes. The carrier gas was Helium (1.0 ml min^{-1}). Quantitative calibration was achieved by running a series of fatty acid methyl esters at known concentration and integrating the peak area in the resulting chromatograms.

2.5 Fuel properties testing

Delipidated algal hydrochar was analysed by thermal gravimetric analysed (TGA) with combined differential scanning calorimetry (DSC). The hydrochar was heated in air to 800°C (10°C min^{-1}) and held for 20 minutes.

2.6 Germination tests

Three 50 mg samples of delipidated hydrochar were washed sequentially with acetone, ethanol and deionised water. Six 50 mg samples of non-delipidated hydrochar, 3 of which were ground using a ceramic pestle and mortar, were washed with deionised water. The filter papers containing chars were transferred to petri dishes and 20 cress seeds added. Three petri dishes containing moist filter paper and 20 cress seeds acted as a control. Each filter paper was saturated with water and, for 7 days, 5 drops of water was added daily. The shoots and radicles were measured and the germination index (GI) (Tiquia, 2010) calculated as:

$$GI = \frac{RSG \times RRG}{100}$$

where RSG (relative seed growth) is given by

$$RSG = \frac{N_{hydrochar}}{N_{control}}$$

and RRG (relative root growth is given by:

$$RRG = \frac{L_{hydrochar}}{L_{control}}$$

where:

N = number of seeds germinated;

L = mean root length.

3. RESULTS

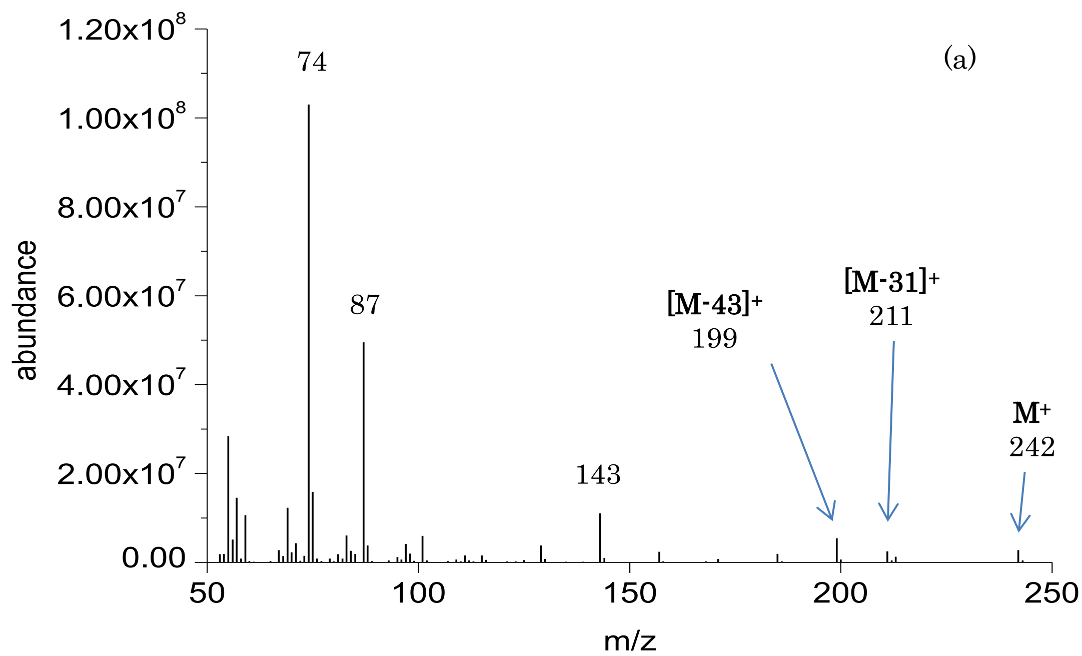
3.1 Oil extraction efficiency

Over the three replicate hydrothermal carbonisation experiments 7.48± 0.08 % of input algal biomass was recovered as oil following hexane extraction of the solid hydrochar product. From the perspective of production of algal biofuels it is important to understand the profile of fatty acids within this extract. To assess this, the extracted oil was transesterified and quantitatively analysed using GC-MS.

3.2 Fatty acid profile of lipid extract

Quantitative GCMS analysis was performed on transesterified extracts from the three replicate HTC preparations. Major analyte peaks are seen at retention times of 9.02, 10.01, 10.10, 10.93, 11.06, 11.90 and 13.94 minutes. Mass spectra at each retention time were characteristic of either saturated or unsaturated fatty acid methyl esters. Representative examples from our data are shown in Figure 1.

In the following, an abbreviation convention of fatty acid identity is adopted in which a fatty acid with 16 carbon atoms and no unsaturation (hexadecanoic acid; palmitic acid) is referred to as 16:0 while a mono-unsaturated acid with 18 carbon atoms (e.g *cis*-9-octadecenioc acid; oleic acid) is referred to as 18:1, etc.



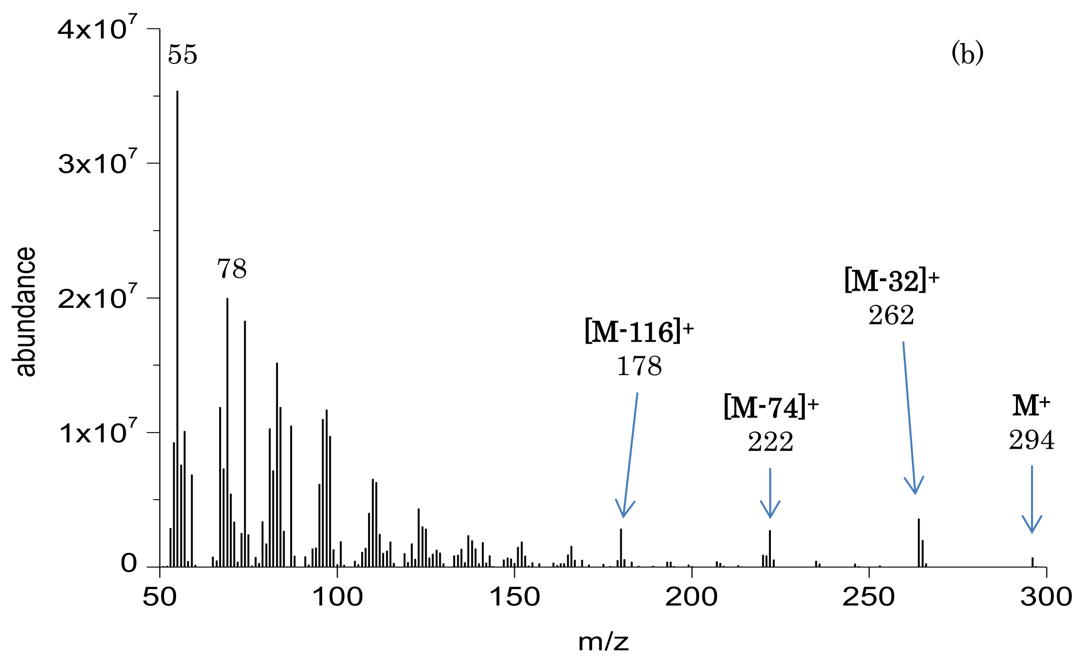
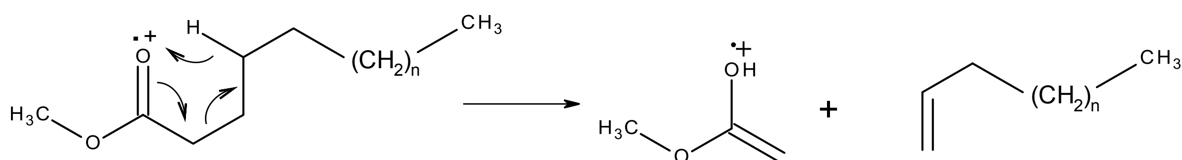


Figure 1: mass spectra for chromatographic peaks at (a) 9.02 min; (b) 10.93 min.

All mass spectra in the GC-MS chromatogram show the McLafferty ion at m/z = 74 characteristic of methyl ester β-cleavage of the molecular ion:



Furthermore, spectra display the molecular ion (M^+) characteristic of the fatty acid methyl ester chain length. On the basis of these molecular ion signals, the spectra in Figure 1 have been assigned to the relevant fatty acid. Further characteristic features of the mass spectra of unsaturated fatty acid methyl esters are the loss of methoxy moiety from the molecular ion ($[M-31]^+$) and loss of C_3H_7 moiety ($[M-43]^+$) through eliminative rearrangement at the hydrocarbon end of the molecule. As the mass spectrometer used here is not capable of detection above m/z = 350, molecular ions of 22:0 and heavier fatty acid methyl esters are not detectable. The assignment of the fatty acid methyl ester at RT = 13.94 minutes as methyl tetracosanoate (24:0) is made on the basis of the characteristic M-43 ion seen at m/z = 339. Other notable features of the mass spectra of the saturated fatty acid methyl esters are the peaks due to $[MeOCO(CH_2)_n]^+$ starting at m/z = 87 (n = 2).

Characteristics of the mass spectra of unsaturated fatty acid methyl esters include the McLafferty ion (m/z = 74) and loss thereof ($[M-74]^+$), loss of methanol ($[M-32]^+$) and a homologous series of fragments resulting from loss of neutral $MeOCO(CH_2)_nCH_3$, with n = 3 resulting in $[M-116]^+$. An homologous series of peaks representing $[C_nH_{2n-1}]^+$ is also evident in the mass spectra of unsaturated fatty acid methyl esters, beginning with the base peak at m/z = 55 (n = 4).

From these mass spectra the assignment of retention time to FAME structure can be made with some certainty; this is outlined in Table 1. Integrated areas of the relevant chromatographic peak have been used to evaluate the relative contribution of each fatty acid to the overall fatty acid profile of the extracted triacylglycerides. This relative composition is also shown in Table 1. The overall yield of the transesterification reaction, based on the mass of lipid extracted from the solid char, was 94.20 ± 10.1 %.

FAME	m/z of M^+	Retention time / min	Relative composition of extracted oil / %
14:0	242	9.02	5.3 ± 2.0
16:0	270	10.10	37.2 ± 0.7
16:1	268	10.01	40.4 ± 6.6
16:2	266	ND	ND
16:3	264	ND	ND
18:0	298	11.06	1.9 ± 0.8
18:1	296	10.93	7.7 ± 3.0
18:2	294	ND	ND
18:3	292	ND	ND
20:0	326	11.90	0.16 ± 0.11
25:0	382	13.49	1.5 ± 0.3

Table 1: assignment of retention time to FAME structure and the relative composition of each component of the extracted oil.

3.3 Thermogravimetric analysis

The TGA and DSC data obtained b heating delipidated algal biochar in air are displayed in Figure 2. The biochar has an ash content of 28%. During heating of the delipidated algal biochar in air a strong exotherm is observed between 150 and 600 °C; integrating this exothermic heat flow yields a calorific value of 8.690 MJ kg^{-1}.

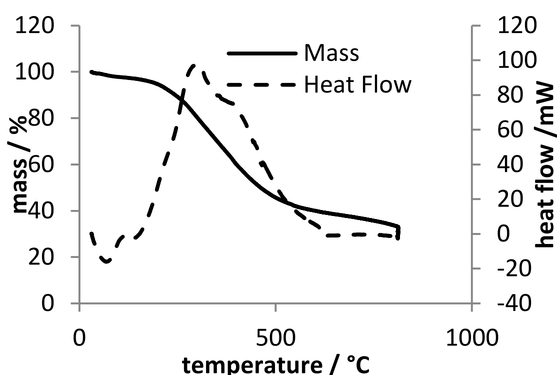


Figure 2: thermogravimetric analysis and differential scanning calorimetry of delipidated algal hydrochar.

3.4 Germination trials

Germination trial results are displayed in Table 2. Although no clear statistical significance is demonstrated between the three conditions, the following observation is worthy of note. Root growth and, consequently, germination index are both greater than 100% in the hydrochar (non-delipidated) conditions. That is, roots in the seeds germinated on non-delipidated hydrochar were longer than in the control. It was also observed that these roots were free of root hairs present in both the control and delipidated hydrochar conditions. Hence, although the internal variability of the trial is too large for statistical differences to be established, a clear observable effect was apparent. Further work is required to increase the statistical power of this test.

	Hydrochar	Ground hydrochar	Delipidated hydrochar
RSG / %	95 ± 12.4	98 ± 7.2	96.7 ± 7.2
RRG / %	105.5 ± 0.6	132 ± 55	74.4 ± 34.4
GI	100 ± 67	130 ± 57	71 ± 37.2

Table 2: germination trial results.

4. DISCUSSION

The recovery of lipids was successful in extracting 6 -7% of lipids from the approx. 4g of *P. tricornutum* HTC biomass. A 20% fatty acid yield is typically expected from this particular strain of algae, although culturing conditions can increase this to around 50%. culturing conditions. Fajardo et al (2007) discuss the optimization of *P. tricornutum* by increasing water content and the use of use of multiple extractions to increase the overall lipid content. Clearly there is scope for optimisaiton of the lipid-recovery protocol.

The fatty acid methyl esters obtained by HTC of *P. tricornutum* contain a high percentage of saturated C16 fatty acids, which is true with many algae. Saturated fats are not an ideal source for biodiesel due to their relatively high melting temperature. The cloud and pour point of putative biodiesels are effected the presence of saturated fatty acids methyl esters and high levels of 16:0 have been observed to affect both of these parameters in winter palm oil. The presence of large amounts of unsaturated fats in the HTC extracted oils mitigates this to some extent.

Jiang and Gao (2004) observed that *P. tricornutum* cultured at 25°C produced less polyunsaturated fatty acids, and as a result were less susceptible to oxidation. In comparison to soybean oil which is currently used as a biodiesel, the total amounts of polyunsaturated fatty acids are not significantly different (Ma and Hanna, 1999). *Botryococcus braunii* which has already been documented for its potential use in biotechnology due to hydrocarbon production, has a saturated composition of 9.85% (dry wt) while other algae strains maintained saturated fatty acid above 32% (Nascimento et al., 2012). The green planktonic microalgae accumulates high oleic acid (18:1) concentrations when hydrocarbon production declines, a profile which is more fitting to crop produced biodiesel (Templier et al., 1984)

The HTC of microalgae produces lipids bound to hydrochar and an aqueous fraction containing soluble products (Valentas & Heilmann, 2011). Subsequent removal of

the lipids produces a delipidated hydrochar. In large scale production disposal or recycling of such large quantities of hydrochar is problematic. A promising value-addition route for this material is through use as a soil amendment. In low quantities hydrochar can be used to improvement soil properties, fertility and as long term carbon storage. However, high concentrations of hydrochar may have a negative impact by effecting plant growth and soil biota (Mohammed, 2013).

The cress seeds in the germination trial showed all control seeds had germinated by day 2. Whereas delipidated and non delipidated chars germination data did not change from day 4, with only 1-2 cress seeds failing to germinate. The chars did not at this point appear to be phytotoxic. The absence of root hairs and increased root length from the char microcosms was apparent. The absence of root hairs during toxic effects has been acknowledged in heavy metals such as copper, zinc and lead (Mahmood, Islam, & Huhammad, 2007). Root length is usually inhibited during such phytotoxicity, and its ability to increase may be indicative of low toxicity levels. Such small effects may have been observed, as both delipidated and non delipidated chars were washed in water prior to extraction and germination. The phytotoxic substances within the chars are mainly water soluble and will significantly reduce germination inhibition (Bargmann et al., 2013). To remove any uncertainty of this data liquor from the washing will be retained for the germination trials. The liquor contains citric acid from the HTC which may also have an adverse effect on such germination trials.

5. CONCLUSIONS

Hydrothermal carbonisation of *P. tricornutum* yielded a good extractible proportion of lipids from the char, which can be improved through optimisation. The remaining char showed low levels of phytotoxicity, which may have been a result of washing chars. The screening microalgae strains for biodiesel production have recommended *Botryococcus braunii* desirable having a high unsaturated lipid content. A high unsaturated value and a low saturated value are required to reduce melting point and provide stability from oxidative stress.

An important area for future work will be to examine char liquors and repeat the process without the washing of chars and analyse the chars by thermal gravimetric analysis (TGA) to reveal volatile carbon and ash content.

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