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Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion

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ABSTRACT

To avoid negative impacts on food production, novel non-food biofuel feedstocks need to be identified and utilised. One option is to utilise marine biomass, notably fast-growing, large marine 'plants' such as the macroalgal kelps. This paper reports on the changing composition of *Laminaria digitata* throughout it growth cycle as determined by new technologies. The potential of *Laminaria* sp. as a feedstock for biofuel production and future biorefining possibilities was assessed through proximate and ultimate analysis, initial pyrolysis rates using thermo-gravimetric analysis (TGA), metals content and pyrolysis gas chromatography–mass spectrometry.

Samples harvested in March contained the lowest proportion of carbohydrate and the highest ash and alkali metal content, whereas samples harvested in July contained the highest proportions of carbohydrate, lowest alkali metals and ash content. July was therefore considered the most suitable month for harvesting kelp biomass for thermochemical conversion to biofuels.

1. Introduction

Climate change and energy security are major drivers for a shift from the use of fossil fuels to renewable energy. A range of renewable energy options exist including wind, solar and tidal but these are intermittent and only suitable for the production of heat and electricity. Biomass is an important part of any renewable energy mix because it is not only capable of providing a stored means of generating heat and electricity, but also of being converted to a range of end-products including transport fuels and platform chemicals.

1.1. Marine biomass

The majority of biomass currently used for biofuel production is from terrestrial sources. Growing biomass on land for fuel can displace other agricultural activities including food production. An increase in the demand for land has caused deforestation and shortages of food result in increased food prices and civil unrest. Solutions to an increased demand for plant products for food, feed, fibre and fuel, include an increase in yields of all crops, and a greater utilisation of marine biomass. For example marine biomass accounts for over 50% of the primary production of global biomass (Carlsson et al., 2007) yet, relative to terrestrial biomass, little is used. Marine biomass comprises of macro and microalgae and both have been associated as potential biofuel feedstocks. In general, microalgae are potential sources of bio-oils whilst macroalgae are potential sources of carbohydrates for fermentation or thermo-chemical based conversions. This paper is concerned with macroalgae as a biofuel feedstock.

Macroalgae are multicellular, macroscopic algae capable of generating more kg of dry biomass m^{-2} $year^{-1}$ than fast-growing terrestrial crops such as sugar cane (Gao and McKinley, 1993). The largest growing macroalgae species are within the phaeophyceae and are termed 'kelps'. In the Atlantic waters surrounding the UK the kelps are primarily members of the laminariales order, growing up to 4 m in length (Hayward et al., 1996). One of the main considerations for the production of biomass for biofuels is the weight of dry feedstock produced. The focus of this research was therefore on *Laminaria digitata*, the most prevalent kelp species growing off the mid-Welsh coastline where the samples for this research were collected.

1.2. Macroalgae biofuels

Previous research on fuels and energy from kelps have primarily focused on the production of methane (Moen et al., 1997), methanol (Horn, 2000) and more recently ethanol (Adams et al., 2009; Horn et al., 2000). A further study investigated the use of a range of macroalgae species as combustion fuels (Ross et al., 2008). However all these trials were conducted on macroalgae harvested at

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one period in the year, and did not consider the seasonal variation which can alter the composition dramatically. Changes in composition is not a new observation, as a study on seasonal variation in *Laminaria* species by Black (1950) demonstrated. Black measured the main carbohydrate compounds (laminarin and mannitol), dry matter and ash content in whole plants and fronds and stipes separately in two environments (open sea and loch), over two years.

Laminarin is the main storage carbohydrate in *Laminaria* species, consisting of a β-(1,3) glucan chain of approximately 25 d.p. with occasional β-(1,6) linkages (Nelson and Lewis, 1974). The other main carbohydrate present is mannitol, which is the alcohol form of the sugar mannose. The dry weight proportion of laminarin varied between <1 and 25% dry weight in *L. digitata*, peaking in October and was absent, or present only at low concentrations, during the winter and spring months (Black, 1950). Mannitol concentration also varied across the seasons, ranging from 3% to 21% of dry weight (Black 1950). The highest concentrations occurred in July and October (open sea) and June and Sept (loch) and the lowest concentrations occurred during winter. The dry matter contents mimicked the laminarin proportion but the ash proportion of *L. digitata* fluctuated conversely, peaking around March and lowest in October (Black, 1950).

Following the study of Black (1950), there has been no subsequent report of the seasonal variation in the composition of UK seaweeds. Seasonal composition has been noted in articles on UK macroalgae e.g. on heavy metal accumulation (Fuge and James, 1973) or radioactive compound accumulation (Nawakowski et al., 2004) but has not been studied further beyond acknowledgement of its occurrence. Given the time that has elapsed and the development of new analytical techniques since the work of Black, it is timely to revisit the topic of seasonal variation in macroalgae composition. In addition as these compositional changes will have an impact on the potential of macroalgae as a biofuel feedstock, the aim of this paper is to investigate the composition and potential for conversion over the year. For example macroalgae can be biologically converted to ethanol or methane through fermentation or anaerobic digestion. Thermochemical conversion methods such as pyrolysis can also be used to produce bio-oil, fractions of which may be used as a direct replacement of fossil fuel-derived diesel. Other energy generation routes could include gasification, combustion and hydrothermal liquefaction.

2. Methods

2.1. Sample collection and preparation

Samples of *L. digitata* were harvested from wild stock at afternoon spring low tides on a rocky outcrop off Aberystwyth beach, Ceredigion, UK (ordnance survey reference SN 581823). Plants were frozen within 1 h of harvesting and subsequently dried at 70–80 °C in a Gallenkamp Hotbox oven (Gallenkamp, Loughborough, UK). Dried material was milled using an A11 Basic IKA mill (IKA, Staufen, Germany) to produce a flour with >90% by weight <1 mm particle size. The flour was then further milled using a SPEX 6770 SamplePrep freezer/mill (Stanmore, Middlesex, UK) for 2 × 1 min pre-chilled with liquid nitrogen to a fine powder.

2.2. Laminarin and mannitol determination

Aliquots of 20 mg ground sample were incubated in duplicate in 2 ml screwcap microfuge tubes, with or without, 1U laminarinase (*Trichoderma* sp., Sigma) in 50 mM succinic acid (pH 5.0) at a final volume of 1 ml. Prepared samples were incubated at 37 °C, 150 rpm for 2 h and the glucose release determined using the glucose determinant assay kit (Megazyme, Bray, Ireland) scaled down to a 1 in 10 volume. A 10 μl aliquot was incubated with 300 μl glucose oxidase–peroxidase (GOPOD) reagent in a flat-bottomed 96 well plate and incubated at 50 °C for 20 min. The plate was read using a μQuant plate reader (Bio-Tek Instruments, Winooski, USA) at 510 nm and the laminarin content of the sample determined from the difference in glucose release between the samples and controls.

A 2% w/w ground *L. digitata* solution was prepared and allowed to equilibrate. An aliquot was centrifuged at 2500*g* for 5 min and 100 μl supernatant mixed with 900 μl of 5 mM H_2SO_4 containing 10 mM crotonic acid (Sigma). This solution was filtered through a 0.45 μm PVDF Durapore filter (Millex-HV, Millipore, Billerica, USA) into 0.2 μl glass-insert vials and analysed using an HPLC system through a Resex ROA-organic acid H^+ column at 30 °C in 5 mM sulphuric acid mobile phase at 0.6 ml min^{-1} (Jasco, Great Dunmow, Essex, UK). A refractive index detector determined peak areas which were compared to calibration and internal standards using the software programme EZChrom Elite Version 3.2 (Scientific Software, Agilent Technologies, Santa Clara, USA) and the concentration of mannitol determined.

2.3. Proximate and ultimate analysis

Moisture content of samples were determined by drying 5.0 g of each sample in a B&T Unitemp oven (LTE, Oldham, UK) calibrated to 105 ± 2 °C overnight. The ash contents were obtained by heating 500 ± 1 mg of each sample to 550 °C for 12 h in an Elite high temperature furnace (Elite Thermal Systems Ltd, Leicestershire, UK) and calculating the proportion retained. The volatile proportion was determined using a thermo-gravimetric analysis (TGA) method representing the initial pyrolysis step. Samples of 5–6 mg were heated from 40 to 900 °C at 25 °C min^{-1} under a nitrogen flow with a 2219 Multitemp II thermostatic circulator (LKB Bromma) and a thermal analyser STA-780 series (Stanton Redcroft). Data was generated by comparing the sample weight against a control crucible which was monitored electronically every 1.67 s. This was electronically recorded using PicoLog recorder software and analysed further using Excel (Microsoft).

The C, H, N and S contents of the biomass were calculated using a CE instruments Flash EA 1112 series elemental analyser in duplicate. The relative percentage of each was determined and the oxygen content calculated by difference and corrected for ash.

Higher heating values (HHV) were determined from the ultimate analysis values using the equation of Channiwala and Parikh (2002). Lower (net) heating values (LHV) were calculated from the HHV using the equation used by the Energy Research Centre for The Netherlands (ECN) (ECN, 2010).

2.4. Metal analysis

Samples of 200 mg were wet digested in HNO_3 in a closed vessel. Metal concentrations were determined using an Optima 5300 DV inductively coupled plasma spectrometer (ICP) with optical emission spectrometry (Perkin Elmer, Cambridge, UK).

2.5. Pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS)

Pyrolysis–gas chromatography–mass spectrometry analysis was performed on a CDS 5000 series pyrolyser connected to a GC-2010 gas chromatograph and a GCMS-QP2010 chromatograph mass spectrometer (all Shimadzu, Kyoto, Japan). Samples weighing 2–4 mg were prepared in duplicate and pyrolysed at 500 °C at a ramp rate of 20 °C per milli-second with a hold time of 20 s. Separation occurred on an Rtx 1701 60 m capillary column, 0.25 i.d.,