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Hydrothermal microwave processing of microalgae as a pre-treatment and extraction technique for bio-fuels and bio-products

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ABSTRACT

Microalgae are regarded as a promising source of lipids for bio-diesel production and bio-products. The current paper investigates the processing of microalgal slurries under controlled microwave irradiation. Microwave power was applied to reach temperatures of 80, 100, 120 and 140°C at a constant residence time of 12 min. Microwave irradiation led to disruption of the algal cell walls which facilitated lipid extraction. The influence of inorganic material on microwave heating was assessed for three strains including, Nannochloropsis occulata, Chlorogloeopsis fritschii and Pseudochoricystis ellipsoidea. Mass balances were calculated and showed that the amount of carbon, nitrogen and total mass recovered in the residue was highly dependent on process conditions and algae strain. Hydrothermal microwave processing (HMP) was found to be an effective pre-treatment for hydrothermal liquefaction and extraction of lipids and phytochemicals.

Key words: Microwave, microalgae, hydrothermal, biorefinery, biofuel

1. Introduction

The development of third generation biofuels from microalgae has seen increasing interest in the last decade. Microalgae are able to fixate carbon dioxide from the atmosphere or from anthropogenic sources by photosynthesis more efficiently than terrestrial biomass due to their

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higher photosynthetic efficiency (Brennan and Owende, 2009). The high photosynthetic efficiency of microalgae and their ability to produce lipids led to research investigating the production of bio-diesel by lipid extraction and transesterification to fatty acid methyl esters (FAME). FAMEs can be mixed with diesel and combusted in conventional diesel engines without the need to modify existing engines. Even though there has been considerable amount of research into the production of bio-diesel from microalgae, only few small pilot-scale projects are currently in operation. One of the main issues associated with large-scale production is the supply of sufficient high lipid algal biomass at a reasonable cost. Cultivation of high lipid strains is quite challenging as the strains can be sensitive to environmental influences and are not generally associated with the high growth rates which some high protein or mixed strains can achieve. Another issue of microalgae is the low biomass in water concentrations from cultivation. This is a major concern when a dry feedstock is required in downstream processes, particularly in many conventional lipid extraction techniques such as solvent extraction or bead milling. Recently hydrothermal processing of algae has been proposed and involves processing of a wet feedstock in hot compressed water. Depending on the severity of the reaction conditions, the process is classified as carbonization, liquefaction or gasification with the latter requiring higher temperatures and pressures. Hydrothermal processing does not require a high lipid feedstock as the protein and carbohydrate fraction of algae can also be converted to either a hydro-char, bio-crude or syngas. Hydrothermal processing of algae has significant potential in the manufacture of microalgae derived biofuels and has been recently reviewed (Biller and Ross, 2012).

One issue which is unanswered by a lot of research in hydrothermal processing is the extraction of phytochemicals prior to biofuel production. The extraction of value added compounds is

essential to improve the economics of producing renewable fuels from microalgae and should be considered. Microalgae are a highly promising source of valuable phytochemicals such as pigments, recombinant proteins, mono- and polyunsaturated fats such as omega-3 fats and polysaccharides (Brennan et al., 2012). Limited studies have looked into the extraction of lipids and polysaccharides before further processing into biofuels. Miao et al. (2012) have recently investigated the sequential hydrothermal liquefaction of microalgae with extraction of valuable polysaccharides in the first step with subsequent bio-crude production of the residues (Chakraborty et al., 2012; Miao et al., 2012). Vardon et al. (2012) investigated the solvent extraction of lipids from Scenedesmus prior to hydrothermal liquefaction of the defatted microalgae (Vardon et al., 2012). Hydrothermal processing is a relatively sever procedure where close control of reaction conditions to achieve specific conversion to desired compounds can be quite difficult. Hydrothermal processing as a technique for the manufacture of chemicals is receiving increasing interest. For example the acid-catalyzed hydrothermal production of 5-(Hydroxymethyl)-furfural and levulinic acid from cellulosic biomass has been recently proposed as a renewable source of platform chemicals (Potvin et al., 2011; Raspolli Galletti et al., 2012). Problems can arise however from induction heating, which may lead to unwanted side reactions at localized hot zones resulting in low extraction yields (Tsubaki et al., 2012). Microwave processing has been suggested to provide a more uniform method of heating as the heating occurs due to the rotation of dipolar molecules and vibrations of ions in solution in an electromagnetic field. This mode of heating can reduce residence times, increase reaction rates and provide more accurate control of reaction conditions (Tsubaki et al., 2012). Tsubaki et al. (2012) showed that the addition of halide salts within hydrothermal hydrolysis of cellobiose increases hydrolysis reaction of carbohydrates, results in a reduction of unwanted side reactions

and energy consumption. It is therefore hypothesized that algae, which are naturally high in salts, could prove to be a promising feedstock for microwave processing. Microwave processing could either be used to facilitate extractions of valuable compounds such as polysaccharides or protein, as recently shown by Budarin et al. (2012) or applied as a means to produce a biofuel by microwave-mediated pyrolysis of algae (Budarin et al., 2012; Budarin et al., 2011).

The current study aims to investigate the use of microwaves for the extraction of value added compounds before further processing to biofuels. The influence of inorganic salts on hydrothermal microwave processing is investigated and the process is evaluated as a technique for extraction of valuable compounds as well as pre-treatment for the production of biofuels via hydrothermal liquefaction. During direct hydrothermal liquefaction, proteins in microalgae are broken down to and rearranged to complex nitrogen containing molecules which are found in the bio-crude (Biller and Ross, 2011). This produces a bio-crude with undesirably high nitrogen content which can lead to complications if the fuel is to be upgraded via hydro treatment/hydrogenation and increased NO_x emissions during direct combustion. It has previously been shown that proteins can be hydrolyzed to water soluble amino acids or extracted as proteins to the water phase during subcritical water treatment (Lamoolphak et al., 2006; Sereewatthanawut et al., 2008). If the proteins can be fractionated to the water phase during hydrothermal microwave processing it is expected that a bio-crude of lower nitrogen content can be produced by HTL. Du et al. (2012) performed work similar to this concept by subcritical water pretreatment by conventional heating before flash pyrolysis to produce a bio-oil with fewer nitrogen containing compounds (Du et al., 2012).

2. Materials and Methods

Three microalgae strains were investigated; Nannochloropsis occulata was grown in-house at the University of Leeds. Chlorogloeopsis fritschii (C. fritschii) was grown by the Plymouth Marine Laboratory, UK. The Pseudochoricystis ellipsoidea strain (P. ellipsoidea) was isolated by the DENSO CORPORATION, Japan and has the unique ability to synthesize and accumulate aliphatic hydrocarbons (Imamura et al., 2012). All three strains were freeze-dried before use. Samples were prepared by mixing ~1g of freeze-dried microalgae with 10 mL of deionized water to form a slurry. The low ash containing high-lipid fresh water strain was mixed with 0.1M NaCl to investigate the effects of inorganic salt content on microwave processing. Samples of each strain were prepared in triplicate for each processing temperature used.

2.1 Microwave processing

Algal slurries were processed individually in a sealed quartz reaction vessel of 45 mL volume within a 1.2 kW Milestone StartSynth microwave oven (Milestone Srl, Italy). Samples were heated to 80, 100, 120 and 140°C within 3 min, the temperature was then kept constant for 12 min before a fan was operated to cool the samples. Internal temperatures of the microalgal samples during processing were measured by an IR thermometer and logged on the control display. The energy used during microwave heating was determined through the integration of the power profiles using the computer's inbuilt $\int E/t$ function.

After the samples had been cooled, they were centrifuged for 15 minutes at 3500 rpm to separate the solid biomass sediment from the liquid phases to enable lipid extraction and compositional analysis. The liquid phase was then diluted to 250 mL with deionized water and analyzed for

anions and cations using a DX-100 ion-chromatography analyzer (Dionex, USA). After centrifugation the solid samples were freeze-dried and analyzed for CHNSO content using a CE Instruments Flash EA 112 series elemental analyzer. Around 10 mg of sample were analyzed using a TA Instruments Q5000 thermo-gravimetrical analyzer; temperature was ramped to 105°C in a constant flow of nitrogen to determine the moisture content and subsequently ramped at 10°C/min to 900°C and held for 15 min to obtain the pyrolysis devolatolisation profile. After 15 min air was introduced for 15 min to burn off the fixed carbon and determine the ash content of the biomass. Biochemical and metal analysis of the unprocessed biomass was performed as described previously (Biller and Ross, 2011; Ross et al., 2008). The solid residue was then coated with a thin gold layer before analysis by scanning electron microscopy (SEM) on a Zeiss EVO MA 15 (Carl Zeiss Microscopy, Germany).

2.2 Lipid Extraction and Analysis

Lipids were extracted from the microwaved samples and unprocessed algae by adding 25 mL of dichloromethane (DCM) to the dry biomass/residue and shaken continuously for 45 min in sealed sample containers. Subsequently, Whatman type 3 filters were used to separate the DCM soluble fraction from the defatted solids. Yields of lipids were determined gravimetrically after evaporation of the DCM at room temperature. Size exclusion chromatography of the lipids was carried out on a Perkin Elmer Series 200 HPLC instrument with a Varian PGel column of 30cm length, 7.5 mm diameter, 3μm particle size and a THF mobile phase flow rate of 0.8 mL/min. The lipids were additionally transesterified to FAME using methanol and sulfuric acid. Approximately 2 mL methanol were added to 200 mg of extracted lipids with one drop of sulfuric acid and agitated for 1 hour at 55°C in a shaking water bath. The FAME fraction was separated using pentane and water. The FAME extract was analyzed on an Agilent 5890 GC-MS

using a RTX-1701 capillary column and calibrated using an external FAME standard purchased from Sigma-Aldrich (F.A.M.E. Mix, C8-C24).

2.3 Hydrothermal processing

Approximately 1 g of freeze dried unprocessed and microwave processed algae biomass was mixed with 10 mL of deionized water and sealed in a 1.905 cm outer diameter and 13.5 cm length Swagelok sealed reactor, the remaining headspace contained ambient air. The sealed reactor was submerged to a preheated fluidized sand bath (FSB-3, OMEGA Engineering Ltd, Manchester, UK) at 300°C for a constant residence time of 15 min. Using an internal K-Type thermocouple the time to reach reaction temperature of the reactants was measured to be 2 min. Subsequently the reactor was quenched in cold water, once cooled to room temperature the gases were vented. The reactor contents were decanted and the reactor washed using DCM and deionised water (30 mL each in 15 mL aliquots). The resulting mixture was separated in a separating funnel and filtration to a bio-crude, solids and water phase. The solids and bio-crude were weighed and the water phase diluted to 100 mL with deionised water. Yields of bio-crude and solids were determined and analyzed as described previously (Biller and Ross, 2011).

3. Results and Discussion

3.1 Microwave processing

Three strains of algae were investigated for the purpose of the current research; Nannochloropsis occulata, the high-lipid strain Pseudochoricystis ellipsoidea and the cyanobacteria Chlorogloeopsis fritschii. The strains were analyzed for their elemental, biochemical and inorganic composition and the data is presented in **Table 1**. Nannochloropsis is a marine strain

which was grown in f/2 media and therefore has much higher ash content than the other two fresh water strains; this corresponds to its lower calorific value (CV) of 17.9 MJ/kg as seen in **Table 1**. P. ellipsoidea has the highest CV due to its low ash content and very high lipid content of 67 % measured by the Bligh-Dyer method. This lipid content is higher than reported before for the same strain but the growth conditions used for the strain used for this work are unknown (Satoh et al., 2010). Both Nannochloropsis and C. fritschii have a high nitrogen content of around 9 % which corresponds to their high protein content of 57 and 50 % respectively. The influence of salts on microwave heating was investigated as previously metals and salts have been shown to absorb microwave irradiation and influence reaction rates during microwave processing (Tsubaki et al., 2012). Nannochoropsis contains the largest levels of salts; the concentration of Cl and Na are particularly high. The concentration of Na in Nannochloropsis is around 50 fold higher than for C. fritschii and 1500 fold for P. ellipsoidea. The high lipid strain (P. ellipsoidea) has a low level of inorganics but contains a high K content. Due to the low concentrations of salts in P. ellipsoidea it was decided to process this strain in a solution of 0.1 M NaCl to investigate the effect of inorganics on the hydrothermal microwave processing of microalgae. The three strains were also analyzed for phosphorous content. Phosphorous is an essential nutrient for the cultivation of microalgae; however it is a finite non-renewable resource extracted from phosphate rock and extraction requires high energy inputs (Cordell et al., 2009), therefore the fate of phosphorous during hydrothermal microwave processing and the possibility of nutrient recycling and/or nutrient extraction is investigated and addressed in this study.

Table 1: Proximate, ultimate, biochemical and metal analysis of microalgae feedstock.

	C. fritschii	Nannochloropsis	P. ellipsoidea
H_2O (%)	10.1	9.2	1.2
Ash (%)	3.6	25.7	1.0
C (% daf)	52.2	57.8	61.3
H (% daf)	7.5	8	9.1
N (% daf)	9.8	8.6	2.1
S (% daf)	0.2	n/d	n/d
O* (% daf)	30.3	25.7	27
HHV (MJ/kg)	18.9	17.9	29.4
Protein (% daf)	50	57	25
Carbohydrate (% daf)	44	8	7
Lipid (% daf)	7	32	67
Cl (mg/kg db)	578	76955	10
Na (mg/kg db)	3905	189271	124
Fe (mg/kg db)	692	714	48
K (mg/kg db)	4844	14989	2899
Mg (mg/kg db)	2693	3295	244
P (mg/kg db)	7847	7806	6256

***by difference, n/d=not detected, daf=dry ash free basis, db=dry basis**

Each microalgae sample was processed under hydrothermal microwave conditions at temperatures of 80, 100, 120 and 140°C while P. ellipsoidea was also processed in 0.1M NaCl. The recovered solid fraction was analyzed for elemental composition and ash content and the results are presented in **Table 2**. For C. fritschii around 80-84 % of the total solid was recovered indicating that 20% of the mass resulted in the water phase as water soluble products. The gas produced during microwave processing was not quantified but is assumed to be low as comparable conditions during conventional heating by Garcia Alba et al. (2011) resulted in gas yields of <3 % at 175°C and residence times of 5 and 60 min (Garcia Alba et al., 2011). The processing temperature had no significant effect on the mass of solids or the ash content recovered from C. fritschii. The ash content was reduced from 3.6 % in the initial biomass to

around 2 % in the microwaved samples, indicating that water soluble salts are fractionated to the water phase. The elemental analysis revealed that around 25 % carbon results in the water phase at 80 and 100°C and increased to 31% at the highest processing temperature of 140°C. Fractionation of the nitrogen content of the algae into the water phase would be beneficial as this could potentially upgrade the biomass feedstock for further processing and could also be used as a source of nutrients for microalgae cultivation. HMP of C. fritschii led to a maximum recovery of N to the solids of 91.7 % at the lowest temperature and a minimum of 71.8 % at 140°C. This indicates that at higher temperatures the protein and/or chlorophyll derived nitrogen becomes more soluble in water either by breakdown to more soluble compounds such as hydrolysis of protein to amino acids or by braking cell structures and hence releasing nitrogen compounds to the water phase.

Table 2: Ash content and % carbon and nitrogen recovered to the solid microalgae residue following HMP**.**

C. fritschii	**80°C**	**100°C**	**120°C**	**140°C**
Mass % recovered	83.8	84.0	80.3	82.3
C % recovered	75.6	75.6	73.1	68.9
N % recovered	91.7	82.8	81.0	71.8
Ash %	2.0	2.2	1.3	3.7
Nannochloropsis				
Mass% recovered	49.0	50.3	38.0	27.8
C% recovered	45.8	47.7	36.9	22.3
N% recovered	44.3	43.1	35.8	17.2
Ash %	4.2	4	5.5	6.5
P. ellipsoidea				
Mass% recovered	83.2	80.1	81.9	76.8
C% recovered	87.3	86.3	86.3	82
N% recovered	67.1	62.5	56.5	48.4
Ash %	0.8	1.1	1.3	0

P. ellipsoidea + 0.1M NaCl

Mass% recovered	85.0	83.0	78.7	77.1
C% recovered	89.4	88.5	83.9	80.1
N% recovered	79.1	76.1	58.1	40.8
Ash %	0.4	1.5	0.5	0.5

The results for the marine algae Nannochloropsis differ significantly to those of microwave-processed C. fritschii. A maximum of 50 % of the total mass is recovered in the solid fraction at the lower temperatures of 80 and 100°C, compared to around 80 % for C. fritschii. At 120 and 140°C the recovery is even lower with 38% and 27.8 % respectively. A proposed reason for this is that the high ash content of Nannochloropsis, which is comprised mainly of water soluble salts, is recovered to the water phase. This results in significantly lower ash content in the residue than in the original biomass. The ash contents of the residues range from 4 to 6.5 % compared to 25.7 % in the original feedstock. This is beneficial for further processing, as a high ash content can lead to complications due to chloride stress corrosion and fouling and slagging issues in combustion (Anastasakis et al., 2011). However it is not only the ash that is removed, the carbon recovery is also much lower compared to C. fritschii, the maximum is 48% at the low processing temperatures and is reduced down to 22 % at 140°C. This represents a large loss of carbon into the water phase. The N content of the recovered solids after hydrothermal microwave processing follows the same trend; maximum recovery in the solid is around 44% and this decreases to 17 % at 140°C. This indicates that the majority of the nitrogen is fractionated into the water phase.

For P. ellipsoidea, the mass recovery is remarkably similar to that of C. fritschii at all conditions. The maximum (83.2%) is seen at the lowest processing temperature and the minimum of 76.8 % at 140°C. The mass recovery when P. ellipsoidea is processed in 0.1 M NaCl is only affected

slightly with marginally higher recoveries at 80 and 100°C. The carbon recovery ranges from 87.3-82% when processed in deionized water and 89.4-80.1% when processed in 0.1M NaCl. This represents the highest carbon recovery of the three strains investigated. The ash content of P. ellipsoidea is not significantly affected up to 120°C, however at 140°C the ash content is below the detection limit of TGA analysis, indicating that processing at 140°C removes the ash content completely. The nitrogen content of the solid algal residue is reduced up to 41% when processed in deionized water and 48 % when processed in 0.1 M NaCl at a temperature of 140˚C. For P. ellipsoidea microwave pre-treatment appears to be more beneficial compared to the other strains. Over 80 % of the carbon is recovered and over half the nitrogen is removed, improving the quality of the biomass feedstock for biofuel production by HTL and simultaneously extracting polysaccharides and amino acids to the water phase. This has also been shown by Chakraborty et al. (2012) to be possible in the low temperature hydrothermal treatment of Chlorella sorokiniana (Chakraborty et al., 2012).

The hydrothermal microwave pre-treatment is shown to effectively remove a large fraction of the ash and nitrogen from Nannochloropsis but at the same time removing undesirably high amounts of carbon. The nitrogen removal from C. fritschii is lower but still significant, therefore the advantages and disadvantages of hydrothermal pre-treatment will need to be assessed based on overall mass and energy balances as well the benefits it has on product quality. The influence pre-treatment has on product quality is described in the following sections.

Following HMP, the algal biomass was recovered, freeze dried and visually inspected by SEM. The images of the microwaved cells are included in the electronic supplementary material (**Fig. S1-3**). In summary, it was observed that the cells of C. fritschii in their raw form are linked

together by extracellular material, possibly originating from the freeze-drying process.. This hormogonia is removed even at 80°C but cells were only broken at the highest temperature of 140°C (**Fig. S1 a-c**). SEM analysis of Nannochloropsis revealed that microwaving leads to more compact clustering of cells, where only at 140°C material appears to be torn from the compact clusters **(Fig. S2 a-c)**. Similarly the cells of P. ellipsoidea appear to cluster together after microwaving with hormogonia being removed; individual cells became increasingly less recognizable as the temperature increased (**Fig. S3 a-c**). Generally the data shows the onset of cell disruption which is hypothesized to influence recoveries of different biochemical components.

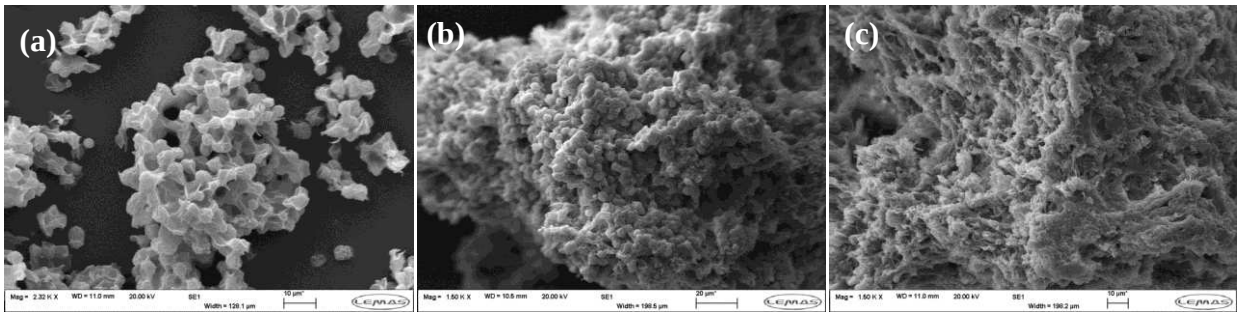

Figure S1: SEM images of untreated C. fritschii (a) Mag=2320, HMP processed at 120°C (b) Mag=1500 and 140°C (c) Mag=1500.

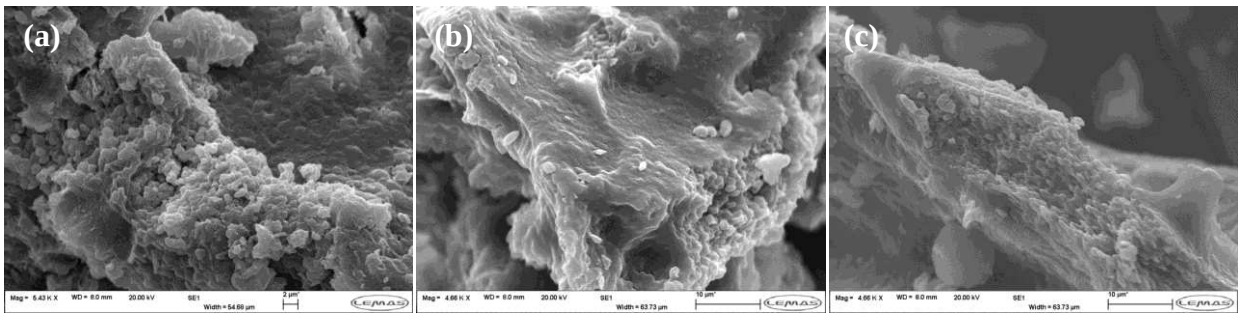

Figure S2: SEM images of untreated Nannochloropsis (a) Mag=5430, HMP processed at 120°C (b) Mag=4660 and 140°C (c) Mag=4660.

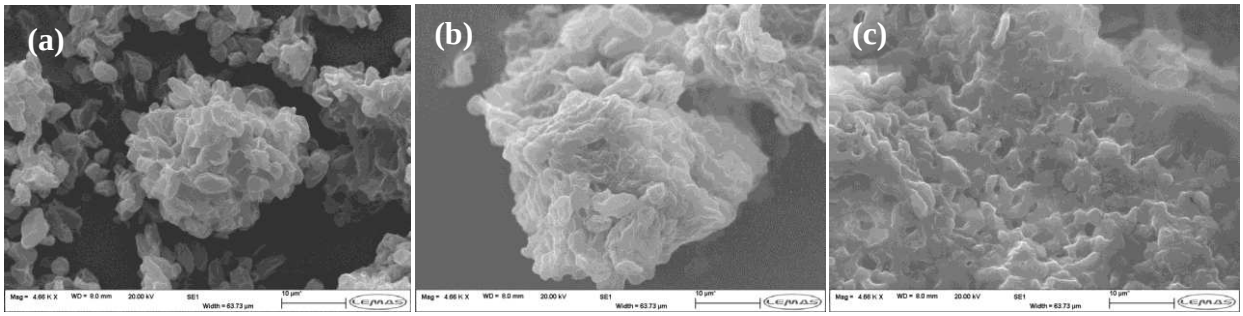

Figure S3: SEM images of P. ellipsoidea (a) Mag=4660, HMP processed at 120°C (b) Mag=4660 and 140°C (c) Mag=4660.

The supernatant after centrifuging the microwaved samples was analyzed for its pH and for anions and cations by ion exchange chromatography (**Table 3**). The fate of these is significant for nutrient recycling for further microalgae growth in a closed loop process, as previously proposed (Biller and Ross, 2012). The levels of Na and Cl in the process water are highest for the marine strain Nannochloropsis followed by P. ellipsoidea processed in 0.1M NaCl while levels are lowest for P. ellipsoidea. Levels of ammonium could only be detected in the C. fritschii sample but levels are much lower compared to those observed during hydrothermal liquefaction (HTL) of the same strain. HTL at 300°C for 1 hour led to concentrations of ammonium of 4750 mg/L compared to around 150 mg/L observed from hydrothermal microwave treatment (Biller et al., 2012). This is due to the much less severe conditions employed in the current study. It is not expected that the protein fraction, containing the majority of nitrogen, is broken down significantly. This is also the reason why there is no ammonium detected in the other strains. The pH of the process water generally decreases with increasing processing temperature for all strains. This could potentially be due to the onset of acid formation by decomposition of simple carbohydrates such as glucose to compounds such as formic, acetic and luvelinic acid. Acid formation was not analyzed in the current study, only the pH was measured, but work by Tsubaki

et al. (2012) showed the effect of increasing organic acid formation from cellobiose under hydrothermal microwave processing resulting in lower pH values at higher processing temperatures and with the addition of halide salts (Tsubaki et al., 2012). This effect is also observed in the current work where the pH values of P. ellipsoidea processed in sodium chloride are lower compared to the samples processed in deionized water.

Table 3: Analysis of water phase after microwave treatment.

		HMP Temperature			
C. fritschii	**(mg/L)**	**80°C**	**100°C**	**120°C**	**140°C**
	Na^+	323	345	357	242
	K^+	174	179	188	140
	NH_4^+	150	147	136	111
	Acetate	47	38	34	57
	Cl^-	-	-	-	-
	PO_4^{3-}	850	915	942	303
	pH	7.97	7.64	7.73	7.42
Nannochloropsis	**(mg/L)**				
	Na^+	7662	8077	8325	8400
	K^+	716	772	787	799
	NH_4^+	-	-	-	-
	Acetate	1550	2240	748	2845
	Cl^-	4080	5248	5373	5628
	PO_4^{3-}	708	930	778	1133
	pH	7.56	7.42	7.66	7.19
P. ellipsoidea	**(mg/L)**				
	Na^+	110	84	103	104
	K^+	258	238	251	232
	NH_4^+	-	-	-	-
	Acetate	-	-	-	-
	Cl^-	54	27	34	31
	PO_4^{3-}	207	118	147	159
	pH	6.62	6.44	6.09	5.62
P. ellipsoidea NaCl	**(mg/L)**				
	Na^+	2122	2286	2183	2295
	K^+	236	262	256	259
	NH_4^+	-	-	-	-
	Acetate	-	-	-	-

Cl^-	3406	3679	3472	3629
PO_4^{3-}	155	155	130	196
pH	6.71	6.66	5.09	5.54

Interestingly the amount of PO_4^{3-} detected in the C. fritschii sample is higher at the lower temperatures and decreases to a third at the highest temperature, leading to the conclusion that if a process-water high in PO_4^{3-} is required, for nutrient recycling or extraction, that mild processing conditions are favorable. Comparing these results with results published previously on HTL at 300°C for one hour, the concentrations are twice as high in the mild microwave processing (Biller et al., 2012). The trend of increasing PO_4^{3-} concentrations in the process water is not observed for the marine strain Nannochloropsis where the concentrations increase by around 30 % at higher temperatures. The values of around 1000 mg/L PO_4^{3-} for Nannochloropsis allow the calculation of a phosphorus (P) balance from the values measured in **Table 1** and indicate that up to 50% of the algal P is recovered to the water phase. This value is between 30-40 % for C. fritschii but only around 15-20 % for P. ellipsoidea. Nannochloropsis is the only strain to exhibit acetate in the water phase, this is the reason the carbon recovery in the solid residue after microwaving is so much lower compared to the other samples. The reason for acetate formation from Nannochloropsis, but not from the other two strains, is not clear but could be due to different type of carbohydrates present in the algae strain. The levels of acetate are comparable to those observed from HTL of different algae strains by Biller et al. (2012) at more severe conditions of 300°C (Biller et al., 2012). Acetate in the water phase can act as a substrate for heterotrophic microalgal growth as previously demonstrated (Bhatnagar et al., 2011; Biller et al., 2012).

3.2 Lipid Extraction and Analysis

Microwave processing has previously been suggested as a technique to facilitate lipid extraction using solvents. Cell disruption by microwaves as seen by SEM analysis (See supplementary electronic material) can lead to much higher recovery of lipids from microalgae than conventional solvent extraction alone. A study by Lee et al. (2010) identified microwave cell disruption as the most simple and efficient disruption method for the recovery of lipids form Botryoccocus sp., Chlorella v., and Scenedesmus sp.. They investigated autoclaving, bead milling, microwave heating (100°C), sonification and osmotic shock (Lee et al., 2010). In the current study simple solvent extraction using dichloromethane was carried out on unprocessed and microwaved samples. The yields of extraction are presented in **Table 4**. For all strains, microwave processing had a large effect on the recovery of lipids. C. fritschii has a very low lipid content and yields of only 0.5 %wt. were observed when the unprocessed sample was subjected to solvent extraction. The recovery increased to 1.4 % after microwave processing at the highest temperature of 140°C. Higher temperatures led to consistently increasing lipid extraction yields for C. fritschii. Untreated Nannochloropsis biomass yielded a 1.6% lipid recovery; this was increased to a maximum of 11.3 % at 120°C. The high lipid strain P. ellipsoidea had the highest yield of lipids of 13.1% when extracted un-treated but increases to about 30-35 % following pre-treatment by microwave processing. Extraction yields increase slightly when processed in NaCl compared to processing in distilled water alone. The differences between the two are small suggesting that the addition of sodium chloride does not have any significant effect on lipid extraction. The increase in lipid recovery is apparent for all three strains with 3-7 fold increases even at the lowest temperature of 80°C. These results confirm the

findings of Lee et al. (2010) who describe microwaving as a low energy intensive method of cell disruption for lipid recovery (Lee et al., 2010).

Table 4: Lipid extraction yields from solvent extraction using DCM of pre-treated microalgae

HMP Temperature	C. fritschii wt% lipid	Nannochloropsis wt% lipid	P. ellipsoidea wt% lipid	P. ellipsoidea 0.1M NaCl wt% lipid
unprocessed	0.5	1.6	13.1	13.1
80°C	0.7	10.6	30.6	31.5
100°C	1	10.5	33.2	33.2
120°C	1.2	11.3	31.4	34.4
140°C	1.4	10	37.5	35.3

Size exclusion chromatography (SEC) was carried out on the samples to investigate if microwave processing has any effect on the structures of the lipid fractions. A representative SEC chromatogram of the unprocessed and HMP lipids of P. ellipsoidea is plotted in **Fig. 1.** It is shown that the majority of lipids are present as triglycerides which represent the largest peak at 6.9 min. This peak was identified to be in the range of Mw 1200, the second peak at 7.8 min is of Mw 420 and represents the free fatty acid fraction of the lipids. There are no changes in lipid profiles observed for the different HMP temperatures. This indicates that no significant hydrolysis of triglycerides to free fatty acids is taking place under HMP.

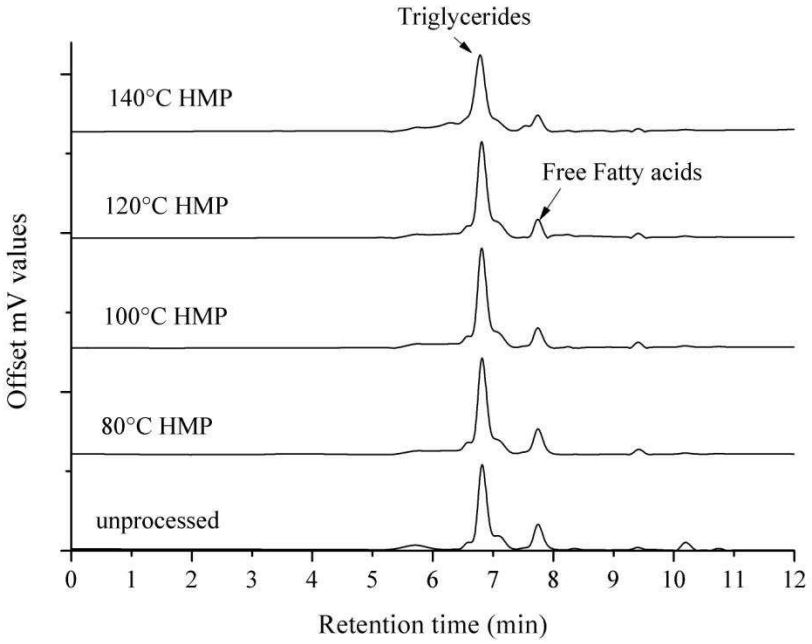

Figure 1: HPLC-SEC chromatogram of P.ellipsoidea indicating the triglyceride and free fatty acid fractions

Additionally the lipids were analyzed for fatty acid composition after transesterification. **Fig. 2** plots the distribution of the fatty acids which were included in the calibration standard. It is known that this algae additionally contains significant amounts of C16:2 and C16:3 which were not included in the analysis (Satoh et al., 2010). The profiles show no change in carbon chain saturation at 80°C HMP. At 100°C around half the C18:3 fatty acids disappear with the remaining fatty acids being present in identical concentrations. At the highest temperature about half the C18:1 fatty acids are also removed and slight increased levels of C18:0 and C18:2 are observed. It is likely that double bonds are removed at the maximum temperature leading to an increase of saturated fatty acids. This leads to the conclusion that polyunsaturated fatty acids can be extracted undamaged with no loss of degree of saturation using HMP at 80°C despite the high sensitivity of omega-3 fatty acids to thermal processing. This is beneficial if the lipids are

extracted as polyunsaturated fatty acids, as these fatty acids are of high commercial value. Additionally it was shown in **Table 4** that the extraction efficiency is already greatly improved at the 80°C HMP temperature.

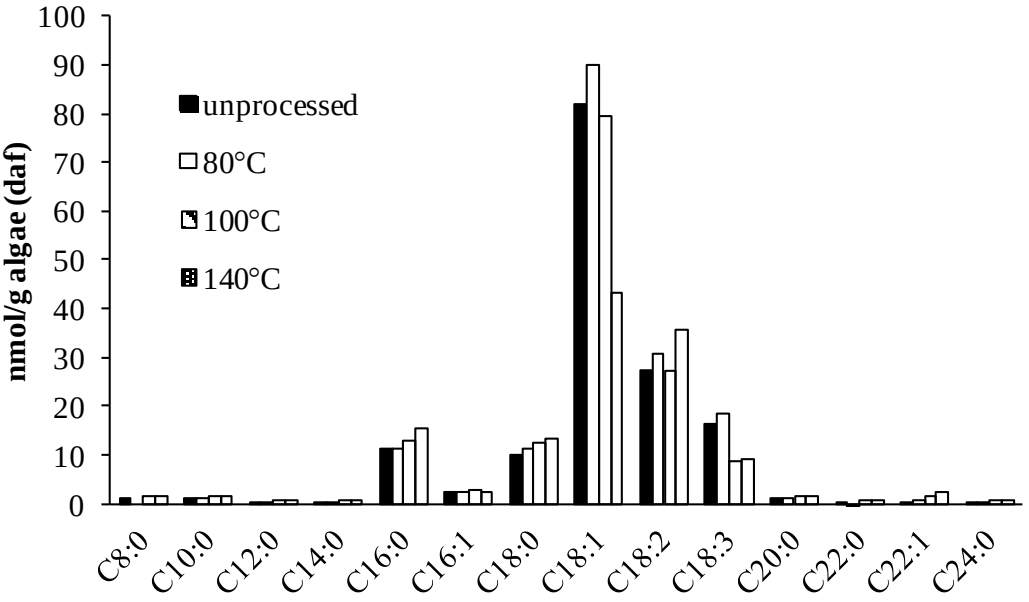

Figure 2: Distribution of fatty acid methyl esters from unprocessed and HMP samples of P. ellipsoidea at 80°C, 100°C and 140°C.

The power required to heat the reactants to the desired temperature and residence time were logged by the microwave reactor and automatically integrated to Wh values to determine the energy used. The Wh were converted to Mj/kg of dry algae to allow comparison to data published in literature and is plotted in **Fig. 3**. It is apparent that more energy is used at the higher temperatures and the trend lines plotted show that the increase is linear. The increase in energy requirement from 80°C to 140°C is around 230-330 % depending on the sample. The low ash and low halide salt containing sample P. ellipsoidea exhibits the largest increase in energy consumption. This sample also has the largest energy requirement at 100, 120 and 140°C of all

samples, indicated by the solid trend line in **Fig. 3**. The second largest amount of energy is required to heat the C. fritschii sample followed by Nannochloropsis and the lowest being P. ellipsoidea processed in 0.1M NaCl. This trend follows the amount of microwave absorbing inorganics, such as halide salts, present in the sample. The energy requirement to heat P. ellipsoidea in pure water to 140°C is 66 % higher than for the sample processed in 0.1M NaCl. This shows that microwave processing of marine algae samples or macroalgae which are also high in ash content is beneficial for two reasons; firstly this technique removes large amounts of the inorganic ash fraction, upgrading the biomass feedstock for further processing. Secondly this approach of heating biomass in salt water takes advantage of the increase in heating by ionic conductance resulting in lower energy requirements to heat the reactants to the desired processing temperature. The values in **Fig. 3** range from 70-270 MJ/kg algae while the original feedstock only contains a maximum of 29 MJ/kg. Clearly it appears to be energetically unfeasible to process microalgae using HMP regarding these values. However it has to be considered that this is a laboratory study with the main objective of investigating the effects of HMP rather than energy usage. The energy requirement can be reduced significantly be various parameters such as solids loading, residence time and total reactant mass in the microwave. Continuous processing in the reactor also deserves investigation as this can greatly decrease the applied power. Nevertheless the current study compares favorably to other pretreatment methods such as sonication (132 MJ/kg algae), high-pressure homogenization (529 MJ/kg), bead milling (504 MJ/kg) and other microwave processing studies (140-420 MJ/kg) (Lee et al., 2012).

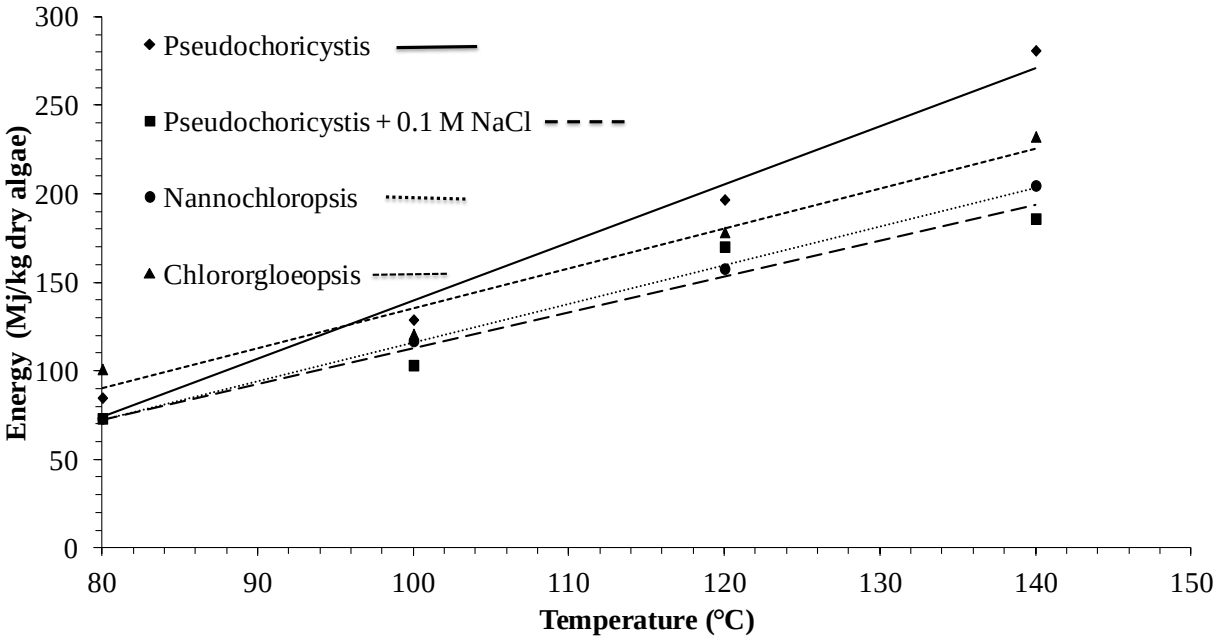

Figure 3: Specific energy requirement to heat samples to desired temperature at constant heating rate and residence time.

3.3 Hydrothermal processing of pre-treated algae

Hydrothermal microwave processing was evaluated as a pre-treatment technique for the production of a bio-crude by hydrothermal liquefaction. Similar work was carried out previously by Miao et al. where conventional heating was used to hydrothermal pre-treat Chlorella before liquefaction for bio-crude production. Their investigation showed positive results as valuable polysaccharides were extracted in the first step, reducing the overall energy requirements and producing a lower amount of unwanted solid residue in the process (Miao et al., 2012). In the current work the microwave processing residues were subjected to HTL at 300°C for 15 min and compared to unprocessed samples. The results presented in **Table 5** show that the yields of bio-crude did not increase significantly for Nannochloropsis and C. fritschii. It should be noted that

the yields were calculated on a dry ash free basis, therefore the yields of Nannochloropsis on a as-received basis would be much higher for the pre-treated samples than for the untreated sample as this exhibits an ash content of 25% compared to ~5% for the microwaved samples (see **Table 2**). One of the aims of pre-treating the algae was to reduce the amount of nitrogen in the final bio-crude product which does not occur to any significant extent for C. fritschii or Nannochloropsis. The samples of C. fritschii at 140°C did show a decrease of nitrogen content of almost 1 % however the oxygen content increased leading to a lower HHV. Apart from the higher yields of bio-crude on an as-received basis for Nannochloropsis, microwave pre-treatment of Nannochloropsis and C. fritschii prior to HTL does not appear to be particularly beneficial. However the results for P. ellipsoidea are more positive; the amount of nitrogen in the bio-crude decreases consistently with increasing pre-treatment temperature. This can be expected from the mass balance presented in **Table 2**, as more nitrogen is fractionated into the water phase. The nitrogen content is reduced from 1.7 % to 0.6% at 140°C pre-treatment temperature. Additionally the yields of bio-crude increase from 33.4 % to a maximum of 49.5%, this is most likely due to some initial hydrolysis reactions of the algae compounds which are more easily converted to bio-crude during HTL. The HHV was increased by almost 10 MJ/kg as a result of the decreasing amounts of oxygen in the bio-crude. This was reduced from 20% to 10.5%. These results show that the bio-crude quality is increased significantly when the P. ellipsoidea samples are pre-treated by microwave irradiation with minimum energy requirements.

4. Conclusions

Hydrothermal microwave processing (HMP) has been demonstrated to be a low energy intensive processing method for microalgae. It is especially suited for high ash, marine strains as the

inorganic salts act as microwave absorbers. Microwave pre-treatment was shown to increase lipid recovery by 3-7 fold and produce a bio-crude of increased quality following hydrothermal liquefaction. Even at mild processing conditions, large amounts of the nutrients such as P and N are recovered in the water phase reducing the levels in the residue. Due to the mild processing conditions, the extracts such as polysaccharides and fatty acids are undamaged allowing the simultaneous extraction of bio-products and nutrients leaving a residue which following further processing results in a biofuel of increased quality.

Table 5: Bio-crude yields, elemental composition and HHV from the hydrothermal liquefaction of pre-treated algae.

		Bio-crude yield	Ultimate analysis (%)					HHV
Sample	**HMP Temp**	**(% daf)**	**C**	**H**	**N**	**S**	**O**	**(MJ/kg)**
Nannochloropsis	**raw**	25.7	72.3	10.6	4.5	0.0	12.6	40.8
	80°C	24.6	73.1	10.2	4.9	0.0	11.9	40.4
	100°C	29.4	67.6	10.2	4.7	0.0	17.5	39.1
	120°C	22.5	70.2	9.1	4.9	0.0	15.8	38.2
	140°C	26.5	73.2	10.8	5.1	0.0	10.9	41.3
C. fritschii	**raw**	20.5	69.1	8.9	5.5	0.0	16.5	37.7
	80°C	18.8	68.8	9.1	6.9	0.0	15.2	37.7
	100°C	18.0	67.3	8.8	6.0	0.0	17.9	37.0
	120°C	19.7	65.5	9.1	6.4	0.0	19.0	37.0
	140°C	23.9	63.6	8.1	4.7	0.0	23.6	35.3
P. ellipsoidea	**raw**	33.4	72.3	6.1	1.7	0.0	19.9	35.0
	80°C	43.0	74.0	11.6	0.8	0.0	13.6	42.9
	100°C	47.4	74.7	11.6	0.9	0.0	12.8	43.1
	120°C	49.5	77.1	11.0	0.8	0.0	11.1	42.9
	140°C	44.1	76.7	12.2	0.6	0.0	10.5	44.4

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