Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp

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Title

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Abstract

The biomass of halophytic microalga *Tetraselemis* sp. with 16%w/w solids was converted into biocrude by a hydrothermal liquefaction (HTL) process in a batch reactor at different temperatures (310, 330, 350 and 370^{o}C) and reaction times (5, 15, 30, 45 and 60min). The biocrude yield, elemental composition, energy density and severity parameter obtained at various reaction conditions were used to predict the optimum condition for maximum recovery of biocrude with improved quality. This study clearly indicated that the operating condition for obtaining maximum biocrude yield and ideal quality biocrude for refining were different. A maximum biocrude yield of ~65wt% ash free dry weight (AFDW) was obtained at 350^{o}C and 5min, with a severity parameter and energy density of 5.21 and ~35MJ/kg, respectively. The treatment with 45min reaction time recorded

~62wt% (AFDW) yield of biocrude with and energy density of ~39MJ/kg and higher severity parameter of 7.53.

Keywords: Biocrude; Energy; Hydrothermal liquefaction; Microalgae; *Tetraselmis* sp.

1. Introduction

Currently, the majority of the energy requirements of the World are met through fossil fuels. Over dependence of fossil energy has resulted in volatile crude oil prices and contributed significantly to environmental pollution leading to global warming (Huber *et al*., 2006). Renewable and sustainable alternative energy sources such as microalgal biomass show great potential to complement fossil fuels to meet our energy needs in the future. Compared to terrestrial biomass, these microalgae grow faster and they do not compete with food crops for arable lands and fresh water (Patil *et al.,* 2008). Microalgae can be readily converted into transportation liquid fuels and chemicals using advanced thermal conversion technologies such as hydrothermal liquefaction (Elliott, 2007).

Hydrothermal liquefaction is a promising reaction that involves the conversion of biomass in sub-critical water to hydrocarbons (Peterson *et al*., 2008), usually referred to as biocrude or bio-oil. Hydrothermal liquesfaction has been successfully carried out at both sub-and supercritical conditions (Minowa *et al.,* 1995; Faeth *et al*., 2013) with water simultaneously acting as both reactant and catalyst. The process has many advantages over other conversion technologies e.g. pyrolysis (Biller *et al.,* 2011). Under subcritical conditions the depolymerisation and repolymerization of complex molecules occurs to produce biocrude (Kruse and Dinjus, 2007). Significantly, unlike other thermal conversion technologies, HTL obviates the need for drying of the feedstocks for producing biocrude.

HTL process results in the production of biocrude along with solid residue, gas and aqueous fractions. The primary product, biocrude is upgradable to liquid transportation fuels. The gas phase containing >98 % CO_2 and <2% hydrocarbon gases, and the aqueous phase rich in major, secondary and micronutrients can be recycled to the algae cultivation ponds to offset CO_2 and nutrient requirements (Frank *et al.*, 2013). Effective utilisation of the HTL product streams for microalgae

cultivation will assist with improving technical and economic sustainability of this process. Previous studies highlight the possibilities of using the nutrient rich HTL aqueous phase as a recycled growth media for the cultivation of microalgae after suitable dilution (Jena *et al*., 2011b; Biller *et al*., 2012).

The biomass obtained from different microalgal strains were successfully converted into biocrude through HTL, which include *Spirulina* sp. (Ross *et al*., 2010), *Nannocloropsis* sp. (Elliott *et al*., 2013), *Dunaniella tertiolecta* (Minowa *et al*., 1995), *Chlorella vulgaris*, *Porphyridium cruentum* (Biller and Ross, 2011), and *Desmodesmus* sp. (Alba *et al*., 2012). There is general agreement that different microalgal species can be converted into a complex mixture of biocrudes with higher heating values (HHV) that are comparable to conventional petrocrude oils. Microalgae strains characterised with higher biomass productivity are found to be beneficial for the production of biocrude. The halophytic microalga *Tetraselemis* sp. used in this study was known for its higher biomass production potential under various salinity levels ranging from saline to hypersaline conditions (Fon Sing *et al*., 2014).

Previous studies on HTL of algae were conducted either in batch or continuously operated HTL reactors under similar processing conditions (Biller and Ross, 2011, Jazrawi *et al*., 2013). One of the prime objectives of previous HTL studies was to optimise the reaction conditions i.e. temperature, holding time and solids content for maximum biocrude recovery (Valdez *et al*., 2012, Jena *et al*., 2011a). The maximum biocrude recovery was mostly achieved with feedstocks containing ~20 % w/v solids at subcritical conditions with the use of water as a reaction medium (Brown *et al*., 2010). Maximum biocrude yield was recorded at higher temperatures close to critical point of water, with significant reaction times of 60min (e.g. Biller *et al*., 2011). Recently the feasibility of achieving maximum biocrude recovery at relatively short reaction times e.g. 1 to 5 min have been demonstrated by Faeth *et al.*, (2013) and Alba *et al*., (2012).

The presence of heteroatoms such as N, O and S in the algal biocrude poses major challenges for the downstream refining process to produce fuel products (Barreiro *et al*., 2013b, Alba *et al*., 2012). Elliott *et al*., (2013) recently reported that heteroatoms in biocrude could be removed and/or reduced by hydro-treatment in the presence of suitable catalysts. Furthermore, the economic profitability of HTL processes are dependent on the quality of feedstocks and the process conditions which will result in maximum biocrude yield and less hydrocarbon loss in gas, aqueous and solid fractions (Alba *et al*., 2012, Brown *et al.*, 2010). Comparison of yield of biocrude, gas, aqueous and solid fractions reported in previous studies is difficult due to variation in feedstock biochemical composition, reactor configuration and process conditions used by the researchers. The maximum biocrude yields obtained reported in previous studies with various microalgal strains at different reaction times 60min were mostly in the range 15-52wt% (AFDW) (Valdez *et al*., 2012, Minowa *et al*., 1995). Faeth *et al*., (2013) recently reported 66wt% of biocrude yield from *Nannochloropsis* sp. at 600^{o}C and 1min holding time. This fast HTL looks attractive as the reaction time was very short. However, more studies are required to assess the economic viability of this fast HTL technology.

A review of the scientific literature provided limited information on HTL of *Tetraselmis* sp. for biocrude production. Interestingly, *Tetraselmis* sp. was among the most productive biomass producers (0.28 to 0.32g/L/day) of the thirty strains of microalgae screened by Rodolfi *et al*., (2009). *Tetraselmis suecica* was one among the eight microalgae strains investigated for biocrude production by Barreiro *et al*., (2013a) who reported 29.4wt% and 45.6wt% of biocrude yield at 250^{o}C and 350^{o}C, respectively.

This study provides a detailed investigation on the effect of reaction temperature and holding time on the hydrothermal liquefaction of *Tetraselmis* sp. for the production of biocrude. The main aim of the study was to optimise the operating conditions of HTL for the maximum recovery of biocrude from *Tetraselmis* sp. and assess its quality.

2. Materials and Methods

2.1 Analysis

A halophytic microalga, *Tetraselmis* sp., used for this investigation was grown and cultivated in open raceway ponds owned and operated by Muradel Pty Ltd in Karratha, Australia. Microalgal biomass after harvesting and dewatering was stored at -8^{o}C. The HTL process was performed at the Aban Infrastructure Pvt Ltd, Biotechnology division pilot plant in Chennai, India. To transport the biomass to India, the biomass was freeze dried at a temperature and vacuum pressure of -48^{o}C and 0.133 mBar, respectively, using 18 L Labcono FreeZone drier.

The biochemical and elemental composition (C H N S O) of microalgal biomass was determined prior to liquefaction. Protein, carbohydrate and lipids were estimated following the methods of Lowry *et al*., (1951), Dubois *et al.,* (1956) and Folch *et al.,* (1956), respectively. The CHNS composition was determined according to the ASTM D-5291 method using a VarioEL III Elemental Analyser System, GmbH. The oxygen content was calculated by subtraction from the combined mass of carbon, hydrogen, nitrogen, and ash. To estimate the ash content ~ 300mg of sample was incinerated at 450^{o}C for 4h in a silica crucible using a muffle furnace. All measurements were in triplicates and the mean values were reported.

The data from the elemental composition were used to estimate the higher heating value using Eq. (1) proposed by Channiwala and Parikh, 2002;

$$HHV\ (MJ/kg) = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151N - 0.0211A \quad (1)$$

where C H N S O and A represents the mass of carbon, hydrogen, nitrogen, oxygen, sulphur and ash, on a dry weight basis.

2.2 Hydrothermal liquefaction

Hydrothermal liquefaction experiments were carried out in a 1L batch high-pressure reactor made of Inconel. Based on literature data the liquefaction experiments were investigated at various operating temperatures (310, 330, 350 and 370^{o}C) and reaction times (5, 15, 30, 45 and 60 min) using

microalgal biomass feedstock containing 16%w/w solids. For each run, the reactor was charged with 16%w/w solids, which was obtained by mixing 60g of dry microalgal biomass with 300ml of seawater, after which the reactor was sealed. The reactor was then heated to the desired temperature using an electrical heating jacket (~20^{o}C heating rate), and held at the target temperature (±4^{o}C) for the predesigned reaction time period. In all the experimental runs, the reactor was continuously stirred by a specialised magnetic drive impeller type agitation device at 300rpm to ensure proper mixing. At the completion of the reaction holding time the heating was switched off followed by cooling to ambient temperature by passing tap water via cooling coils.

The gas phase was collected in Tedlar bags via the reactor gas vent after cooling the reactor to ambient temperature. The gas phase yields were quantified by the difference in final and initial weights of the Tedlar bag and expressed as a weight percentage relative to the amount (dry weight) of microalgae feed used for the HTL process. The reactor was then opened and the reaction mixture was transferred to a separating funnel. The reactor wall and stirrer was then washed with 50mL each of dichloromethane (DCM) and deionised water, to recover the residual reaction product from the reactor surfaces. The washed fractions were added to the rest of the reaction mixture in the separating funnel. A schematic of the product recovery and separation procedure is shown in Figure 1. An equal volume of DCM to the reaction mixture was added into the separating funnel for phase separation and biocrude extraction from the solid and aqueous fractions.

The solid residue that was separated from the aqueous phase was washed 3 times with DCM (50mL) for maximum recovery of biocrude. The DCM fractions containing biocrude were pooled and the solvent was vacuum evaporated at ~40^{o}C to purify the biocrude. The biocrude yield was reported as wt% on an ash free dry weight basis. The mass of the solid residue and dissolved aqueous phase were quantified after drying in a hot air oven at ~100^{o}C for about4h and 8h, respectively, to eliminate residual solvent and water. This was based on the work of Theegala and Midgett, (2012). The same

procedure as described in section 2.1 was followed to determine the ash content. All experiments for 310 and 330^{o}C treatments were conducted as single batch runs whereas duplicate runs were performed for the experiments at 350 and 370^{o}C, at different reaction times (5, 15, 30, 45 and 60min). Each sample was analysed for elemental composition and HHV as previously described previously in section 2.1. The energy recovery (ER%) was calculated using Eq.(2) based on previous work (Alba *et al*., 2012, Miao *et al*., 2012).

ER=(HHV(MJ/kg) of product*Mass(g) of product)/(HHV(MJ/kg) of feed * Mass of feed(g)) (2)

The equation does not consider the external work applied for heating the reactor system but by relating the higher heating values and weight of biocrude to that of initial microalgae fed to the reactor (Biller and Ross, 2011). The carbon and nitrogen recovery (%) was estimated using the elemental mass balances across the reactor. Approximately 300mg of ash fraction of the resultant biocrude obtained from incineration at 450^{o}C, 3h, was wet digested in nitric acid, followed by metallic analysis using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Agilent 7500 series.

The severity parameter (equation 3) was also incorporated in the product analysis with respect to the experimental operating parameters.

$$Ro = \int_{t1}^{t2} \exp[(Tr - Tb)/14.75]dt \quad (3)$$

where t_1 and t_2 is the initial and final holding time, respectively, T_r is the reaction temperature ^{o}C, T_b is base temperature, assumed here to be 100^{o}C. This equation has been used previously as a metric for temperature and time effects in processing microalgae (Faeth *et al*., 2013) and in sewage sludge (Yanagida *et al*., 2010). It combines the effect of the significant state variables (temperature and reaction time) into a single parameter, and correlates with data obtained at different operating conditions.

Results and Discussion

3.1. Feedstock analysis

The elemental and biochemical composition of *Tetraselmis* sp. used in the present study is presented in Table 1. In the reported work, the microalgal biomass was processed without the addition of any catalysts. Compared to the other microalgal strains the carbon content of *Tetraselmis* sp. observed in the present study was low and therefore, the higher heating value of this strain was less than the other micoralgal strains reported previously (Table 1). However, *Tetraselmis* sp. used in the reported study had a higher hydrogen-to-carbon ratio (H/C) atomic ratio of 1.94 compared to the H/C ratios recorded for *Nannochloropisis oculata*, *Chlorella vulgaris*, *Porphyridium cruentum*, *Spirulina* sp. and *Desmodesmus* sp. (Table 1). The higher the H/C ratio the higher is its value as it leads to more energy release upon combustion of the feedstock.

3.2. HTL product yields

The yield of HTL products obtained from *Tetraselmis* sp. is represented in Figure 2. The treatment at 310^{o}C with reaction times ranging from 5-60min resulted in 40-46, 22-27 and 13-19wt% yields of biocrude, aqueous phase and solid residues, respectively. In these treatments, the reaction time of 5 and15min resulted in ~40wt% biocrude yield and treatments above 30min showed marginal increases in biocrude yield. There was no substantial effect with the various reaction conditions on the solid residue and aqueous phase, especially at low temperatures. This was attributable to the fact that at low temperatures, hydrolysis is the predominant reaction (Alba *et al*., 2012), which leads to high yields in solid residue and aqueous phase. At low temperatures, the organics simply dissolve and remain in the aqueous phase with less conversion to other product fractions such as biocrude and gas.

The treatments at 5 and 15min reaction time at 330^{o}C showed no difference in yields for all product fractions. However further increase in reaction times >15min led to substantial increase in biocrude yields up to 60wt%, confirming subsequent cracking and polymerization reactions. There were

substantial increases in biocrude yields with increased reaction times between 30 and 60min with corresponding decreases with the aqueous phase yield, indicating direct conversion of dissolved organics in the aqueous phase to biocrude. HTL treatment at 350^{o}C with a 5min reaction time resulted in a 65wt% yield of biocrude when compared to 54, 51, 45 and 41wt% biocrude yields obtained at 15, 30, 45 and 60min reaction times, respectively. Interestingly HTL treatment at 370^{o}C with a reaction times of 5 and 15min resulted to decrease in biocrude yield (37-42wt%), which was consistent with the increase in measured gas phase yields. The decrease in biocrude yields at higher temperatures i.e. 370^{o}C was attributed to production of gaseous products (Figure. 2d). Based on this data it was concluded that high temperatures favour gasification of organic matter which led to high gas yields with corresponding low yields in other product fractions like biocrude. This was mostly due to the formation of lighter and more volatile hydrocarbons that were neither retained in the biocrude phase nor in the higher molecular weight compounds presents in the solid phase (Brown *et al.*, (2010).

The trends in product yields observed in the present study were similar to most previously reported studies. For instance, Alba *et al.*, (2012) obtained biocrude yields of 8.6-40.5wt % with corresponding yields in aqueous phase (21.6-30.5wt%) and solid residues (66.1-15.5wt%) at 175-300 ^{o}C. They also observed an increase in yield of biocrude (40.7-49.4wt%) with increase process temperatures up to 375^{o}C with a corresponding decrease in both solid residue (9.2-15.5wt %) and aqueous phase fraction (15.2-30.5wt%). They also obtained increase in gas yields with increased reaction temperature and reported a maximum gas yield of 47.4wt% at 450^{o}C. Jena *et al.*, 2011a and Biller and Ross, 2011 reported ~28 and 30wt% gas yields for *Spirulina* sp. and *Nannohloropsis* sp., respectively, in the HTL run carried out at 350^{o}C with 60min reaction time. The gas yield is comparable to the 33wt% gas yield obtained in the present study at the same operating condition (350^{o}C, 60min reaction time) used in previous studies.

In contrast to most past research investigations, the yields of solid residues obtained in this study were generally higher >10wt%. For example Jena *et al*., (2011a) reported decreases in solid residues from 22 to 6wt% at 300^{o}C, to 5 and 5.7wt% at 350 and 380^{o}C, respectively. In addition, Biller and Ross, (2011) obtained <10wt%of solid residues following processing of different microalgae at 300^{o}C, 60min, Alba *et al*., 2012 reported 9.2wt% solid residue at their optimum process condition (375^{o}C, 5min) based on biocrude yield. Valdez *et al*., (2012) reported 8 and 3wt% of solid residue at 250^{o}C and 350^{o}C, 60min. The difference in resultant of solid residues could be attributable to presence of more inorganic matter in the biomass sample in the present investigation. In this study, the amount of solid residue fractions varied less with further increases in reaction times beyond 5min at 350^{o}C (see Figure 2c). Suggesting that majority of organic matter is converted into biocrude and other product fractions within 5min of the HTL treatment.

Currently, HTL research is mostly focused to enhancing biocrude yield at the shortest possible reaction time (Faeth *et al*., 2013, Jazrawi *et al*., 2013). As processes with short holding time of few minutes may require smaller reactor volumes, thus lower capita cost commercial operations (Valdez *et al*., 2012). The data obtained in this study confirms that biocrude yields can be produced at very short reaction times (5minutes), which is economically more advantageous when operating at subcritical conditions. Alba *et al*., (2012) reported biocrude yields of 49wt. % from *Desmodesmus* sp. treated at supercritical condition (375^{o}C, 5min). Similarly various algal strains used by Barreiro *et al*., (2013a) processed at 375^{o}C, 5min recorded biocrude yields of 45.6-58.1wt%. Faeth *et al*., (2013 Faith *et al*., (2013) reported a biocrude recovery of 66wt% from *Nannochloropsis* sp processed at one minute holding time but also at supercritical temperature (600^{o}C). From the results obtained in the present investigation, it is concluded that higher yields of HTL biocrude from *Tetraselmis* sp can be obtained using short reaction times at subcritical conditions of 350^{o}C. The underlying chemistry for high yields of biocrude at short reaction time in HTL treatments could not be fully ascertained due to the complexity of depolymerisation and repolymerization reactions. However, one of the possible

explanations for higher biocrude yield at short holding time could be due to the quick release of the intracellular content of the microalgae leading to sharp decrease in solid residue and increase in high biocrude yield (Valdez *et al*. (2012).

It is difficult to adequately compare HTL product yields with other literature studies due to varying biochemical compositions in the individual feedstocks, reaction conditions and reactor configurations. Even though the lipid content in the alga used in the present study was low, the HTL biocrude yield obtained was significantly higher than most previously reported yields. Earlier studies reported maximum biocrude yields of 34.3 and 35wt% from *Nannochloropsis oculata* and *Chlorella vulgaris*, respectively (Ross *et al.,* (2010), whereas 39.9 and 46.5wt% biocrude yields were reported for *Spirulina* sp. and *Desmodesmus* sp. (Jena *et al*., 2011, Alba *et al*., 2012). All the reported biocrude yields in the previous investigation were obtained under the same operating condition i.e. 350^{o}C and 60min reaction time, with little variations in the solid concentration. In this study 41wt% biocrude yield was obtained at the same reaction condition (350oC, 60min) which is in agreement with the biocrude yield reported in previous research studies. This investigation established a proof of concept for obtaining higher biocrude yields from *Tetraselmis* sp. through efficient HTL with a reaction time of 5min at 350^{o}C.

Apparently biocrude yield significantly impact life-cycle analysis (LCA) data. Most LCA sensitivity analysis has indicated many vital areas to improve the energy efficiency of the microalgae-to-biocrude systems. It was suggested that improving the biocrude yield and recovery would substantially improve the energy-return on energy-invested (ERoEI) (Liu *et al*., 2013 and Frank *et al*., 2013). However, the data employed in computing the ERoEI analysis in the previous HTL studies were obtained at longer reaction times i.e. about 60min. It is envisaged that the ERoEI values would be attractive if higher yields of biocrude can be achieved at relatively shorter short reaction times

which could significantly improve the energy-return on energy-invested (ERoEI) for the production process of alga biofuels.

3.3 HTL products analysis: biocrude

3.3.1. Elemental composition

In the reported work the resulting biocrude was dark in colour and free flowing in nature. The HHV of biocrude obtained at the various reaction conditions was 28-39MJ/kg, which was substantially higher than the original feedstock (19.2MJ/kg). The energy density of the biocrude achieved in the present study was in agreement with previous studies (Biller and Ross, 2011, Minowa *et al.*, 1995, Jazrawi *et al.*, 2013). Generally, the biocrude produced had a higher carbon and hydrogen content (67-79%w/w and 6-9.5%w/w), than that of the original microalgae feedstock. The reaction temperature and holding time had a positive influence on the carbon and hydrogen content, which increase in treatment with reaction temperature and time. Similarly, the sulphur content in the biocrude varied, with the maximum concentration occurring at 350 and 370^{o}C process conditions. Despite the variation, the sulphur content in the biocrude was relatively low (<0.9%w/w) compared to other elements such as nitrogen, in agreement with previous reports. The amount of sulphur content for biocrude reported in previous research studies usually were <1%w/w, Brown *et al.*, (2010) and Valdez *et al.*, (2012) reported <0.91 and 0.8%w/w, respectively. The nitrogen content of the biocrude was undesirably high (~4~6.5%w/w), mostly due to higher protein content in the *Tetraselmis* sp. biomass which is in agreement with published studies (Barreiro *et al.*, 2013a, Faeth *et al.*, 2013, Jena *et al.*, 2011a). However the nitrogen content of the biocrudes was far higher than found in conventional crude oils in agreement with past reports (Jazrawi *et al.*, 2013, Ross *et al.*, 2010, Faeth *et al.*, 2013). Though, the nitrogen content of crude oil has been reported to be as low 1.3%w/w but it is still considered high because of its environmental implications and is potentially poisonous to catalysts employed in the refining processes (Biller and Ross, 2011, Speight, 1999).

Furthermore, the data obtained from the biocrude elemental analysis in the reported work revealed that biocrude produced at short reaction times (5min) are associated with high oxygen content compared to those produced at longer reaction times (60min). Biocrudes associated with a high concentration of oxygen are regarded as poor quality as they exhibit low energy value and poor storage stability (Jena *et al.*, 2011). Although short reaction times (~5min) at 350^{o}C led to maximum biocrude yield, the oxygen content in the biocrude was lower (Figure. 3) at longer reaction times of 45 min. It suggests that conditions for maximum biocrude yield may not be optimal in terms of crude properties desirable for refining. For example Jazrawi *et al*., (2013) reported lower oxygen content in the biocrude at longer reaction time, in their case (5min) than short residence period (3min); Valdez *et al*., (2012) found that short residence times (10min) were more suitable to recover maximum amount of energy in the biocrude, and the recovery were insensitive with further increase in reaction time to 40min. Similar observation were made in the current study. It is generally believed that during HTL reactions oxygen is recovered as H_2O and CO_2 through a series of dehydration and decarboxylation reactions, leading to improved carbon and increase in the H/C atomic ratio within the biocrude (Jena *et al*., 2011a). Based on Figure 3, it could be concluded that longer reaction times are desirable for dehydration and decarboxylation reactions, thus more favourable for improved "quality" biocrude. However, long holding times may be economically unfavourable for commercial production of biocrude in terms of energy consumption. It would be interesting if the maximum yield and best quality biocrude can be obtained at the same reaction conditions i.e. with shorter reaction time of few minutes. The findings from this study have shown that improving the biocrude yield and quality at the same HTL reaction condition seems challenging.

The comparison of the estimated carbon-to-nitrogen and carbon-to-hydrogen ratios of the current work with those of previous studies (e.g. Faeth *et al*., 2013, Alba *et al*., 2012, Biller *et al*., 2011), obtained at optimum conditions showed good similarities (Figure 4a). It was found that improved biocrude quality was linked to higher ratios. In addition, the van Krevelen diagram for different

biocrude (Figure. 4b) indicates that the produced biocrude also exhibit close similarities for the H/C and O/C atomic ratios to that of other algal biocrude, suggesting *Tetraselmis* sp. as a potential feedstock for the production of biocrude.

3.3.2. Metallic constituents in biocrude from *Tetraselmis* sp.

One of the important factors in HTL is the present of metals in the resultant biocrude, and since microalgae, particularly marine strains are typically known to be high in alkali metals, the fate of metals during HTL is a concern. The metallic analysis of the biocrude oil ash fraction revealed the presence of some elements, including aluminium, calcium, copper, iron, potassium, magnesium, manganese, sodium, nickel, and zinc. Their concentrations in the biocrude were 4.3, 16.0, 1.6, 5.9, 9.3, 6.9, 0.4, 160.8, 2.1 and 1.5mg/l, for Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni and Zn, respectively. Most of these elements were reported previously to be present in HTL-algal biocrude and the concentration of these metals was higher than that present in the petrocrude (Anastasakis and Ross, 2011). The metals (e.g. Cu, Fe, K, Mg, and Zn) are trace elements required for microalgae cultivation while Na and Ca are typical media composition, especially for marine strains. Metals in biocrude seemed unavoidable due to adsorption and leaching, especially if operating temperatures are high and the feedstock is rich in metals which also lead to increase in metal/salt content in the biocrude. As a result metals are often found in all types of crude oil and substantial reduction of metal/salt is required prior to refining to liquid transportation fuels in order to avoid issues associated with clogging and fouling. Though, some of these metals (e.g. Cu, Cr, Fe, Mn, Mo and Zn) have been suggested be useful catalysts for upgrading of biocrude (Jena *et al*, 2011a), they could also cause problems, particulary in poisoning the catalysts employed for desulfurization and denitrogenation, increase in gas and coke formation and subsequent decrease in gasoline yields (Speight, 1999). Thus further studies are required to develop novel technologies to reduce salt and metal content in the biocrude for downstream processing operations.

3.3.3. Energy, carbon and nitrogen recovery

Optimum operating conditions leading to high-energy recovery in biocrude is desirable for HTL processes. Higher heating value of the feedstock, reaction temperatures and holding time, are considered important as they significantly influence the biocrude recovery. In this paper the obtained ER was 38-87%, which was within the range reported in the scientific literature earlier (Figure 5) Biller and Ross, (2011) reported energy recoveries of 66, 51.6 and 50.7% in the biocrudes produced from *Nannochloropsis oculata*, *Porphyridium cruentum* and *Spirulina* sp. respectively, at 350^{o}C and 60 min reaction time. Alba *et al*., (2012) reported 73% energy recovery from *Desmodesmus* sp. treated at 375^{o}C for 5min, whereas 55-90% ER was achieved by Brown *et al*., (2010) following processing of *Nannochloropsis* sp. at 200-500^{o}C, 60min. Valdez *et al*., (2012) reported up to 80% energy recovery during liquefaction of *Nannochloropsis* sp at 200-400^{o}C and 10-90min.

The outcome of carbon and nitrogen recovery in HTL products streams is important as it is believed to affect the quality of the products. High carbon and low nitrogen recovery in biocrude is desirable as it affect its quality, in order to reduce NO_x formation during combustion (Anastasakis and Ross, 2011). The carbon and nitrogen recovery details are given in Figure 6. In this work the carbon and nitrogen recovery were 63-88% and 24-53%, respectively, which were found to be within the range of previous reports (e.g. Alba *et al*., 2012, Biller and Ross, 2011). These previous studies obtained up to 70% and 22-40% carbon and nitrogen recovery in biocrude at a reaction temperature of 300-375^{o}C, at 60min holding time. Previous investigations generally agreed that the nitrogen recovery increase with increase in reaction temperature irrespective of HTL feedstock, and the type of reactor system which can be of batch or continuous mode (Jazrawi *et al*., 2013, Valdez *et al*., 2012) but insensitive to reaction time. Higher temperature leads to rapid hydrolysis of proteins in the feedstock to nitrogen compounds e.g. amines, amides, pyroles and indoles through series of decarboxylation and deamination reactions (Biller and Ross, 2011, Toor *et al*., 2012). The increased concentration of

N compounds in the biocrude is undesired and they pose serious challenges in refining operations (Barreiro *et al*., 2013b; Jena *et al*., 2011a).

Therefore upgrading the biocrude is considered essential to eliminate or reduce the nitrogen content before being refined into various distillate fractions to produce liquid transportation fuel products (Brown *et al*., 2010, Ross *et al*., 2010). The recent biocrude upgrading studies using hydrotreating process showed substantial reduction in nitrogen content in the biocrude. However, more research is needed to upgrade the biocrude in a sustainable way to remove impurities. A review of the literature show that biocrude upgrading reaction were carried out at similar operating conditions as used in HTL reactions using specific hydro-treating catalysts e.g. HZSM-5, molybdenum sulphide catalysts with cobalt on a fluorinated support (Elliott *et al*., 2013; Li and savage, 2013). Based on a recent report by Elliott *et al*., on the catalytic hydrotreating of biocrude in a fixed bed reactor, the reduction of heteroatoms such as oxygen, nitrogen and sulphur in biocrude was 76-86, 94-99 and ~99%, respectively, with an increase in carbon and hydrogen content. However, Li and Savage (2013) observed loss of hydrogen atoms which led to low H/C atomic ratios and low HHV during the biocrude upgrading using HZSM-5. Biocrude upgrading is also associated with higher operational costs due to the use of expensive catalysts and frequent fouling of reactor components.

Pretreatment of the microalgae to remove or reduce the protein content prior to HTL is considered beneficial to reduce N content in the biocrude (Alba *et al*., 2012, Biller and Ross, 2011). However, this could have adverse effects in HTL biocrude yield due to loss of desired components in the form of carbon during the pre-treatment step. It is envisaged that after pretreatment, the algae cells will be weak/fragile hence would not require high temperature normally applied for direct liquefaction, suggesting reduction in energy consumption. This pretreatment step helps in maximising biocrude yield at lower reaction temperatures and will reduce energy consumption and high risk associated with operating high temperature-pressure systems. Recent reports have shown that processing of pre-

treated microalgae at much lower reaction temperature led to increases in biocrude oil yield, predominantly due to the initial hydrolysis of the pre-treated microalgae (Miao *et al.*, 2012). Optimisation of process conditions for liquefaction of pre-treated microalgae is necessary to obtain maximum yields of biocrude.

3.4. The Optimum condition

The data presented in Figures 2, 4, 5 and 6 provide a information on biocrude yields and properties over a range of operating conditions. Thus, the optimum condition(s) for biocrude recovery can be ascertained. The process temperatures and reaction times that were investigated were computed as the logarithm value of equation 3. It was found that log Ro increase with increase in reaction temperature and time in agreement with Faeth *et al.*, (2013), suggesting that high biocrude yield at low value of log Ro was preferable in terms of temperature and time. In this study, the maximum biocrude yield of 65% corresponded with log Ro of 5.21, which was found lower in comparison to previous reports (Table 2) that obtained maximum biocrude yield at long reaction time (60min). Indicating that maximum biocrude achieved at short reaction times leads to low log Ro values, long holding periods led to higher log Ro values.

Therefore, it can be concluded that HTL treatment at 350^{o}C and 5 min would be the optimum condition for achieving maximum biocrude yield from the microalga *Tetraselmis* sp used in this study. However, this reaction condition may not result in the ideal quality biocrude quality for refining purposes as different reaction conditions were required to achieve maximum yield and ideal quality biocrude for downstream processing. The composition of the biocrude, particularly carbon and oxygen was different between short and long reaction times. In addition, the data suggest that biocrude yields at low Ro (low severity or low temperature and reaction time) is economically favourable. However, as observed in this study and recent publications, maximum biocrude yield is obtained at high temperature conditions $\geq$ 350^{o}C. This condition is not favourable for the production of desired quality biocrude as it releases more nitrogen compounds from the biomass.

4 Conclusion

HTL of the halophytic microalga *Tetraselmis* sp. in this study indicated that shorter reaction time i.e. 5min at temperature below supercritical point would be ideal to achieve maximum biocrude yield. However, the resultant biocrude did not have desirable composition for downstream refining, thus required further upgrading to reduce heteroatoms and other impurities. HTL treatment with shorter reaction time has many advantages, which include reduction in capital costs and energy consumption. This work further confirms that *Tetraselmis* sp. is an ideal feedstock for biocrude production through HTL and higher lipid content is not an essential criterion for enhanced biocrude recovery.

5 Acknowledgement

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7 Figure captions

Figure 1: HTL of *Tetraselmis* sp. product recovery and separation procedure.

Figure 2: Mass yields in biocrude, solid residue, aqueous and gas phase from liquefaction of *Tetraselmis* sp. at; a: 310^{o}C; b: 330^{o}C; c: 350^{o}C; 370^{o}C; 5-60min.

Figure 3: Comparison of the effect of reaction time on carbon and oxygen content in biocrude with its yield.

Figure 4a: Comparison of biocrude elemental carbon-to-nitrogen and carbon-to-hydrogen ratios of present study with other microalgal species

Figure 4b: van Krevelen diagram for different oils

Figure 5: Effect of operating temperature and reaction time on energy recovery in biocrude produced from liquefaction of Tetraselmis sp at a: 310^{o}C; b: 330^{o}C; c: 350^{o}C; d: 310^{o}C, 5-60min.

Figure 6: Effect of Temperature and reaction time on carbon and nitrogen recovery in biocrude produced at; a: 310^{o}C; b: 330^{o}C; c: 350^{o}C, d: 370^{o}C.

8 Table captions

Table 1: Elemental and biochemical composition of microalgae

Table 2: Properties of biocrude obtained at optimum conditions from hydrothermal liquefaction of different microalgae.

9 Highlights

*Maximum biocrude yield of ~65wt% was achieved

*The maximum yield and quality of the biocrude are independent variables.

*High biocrude yield was favoured by short reaction time.

*Biocrude quality was favoured by long reaction time.

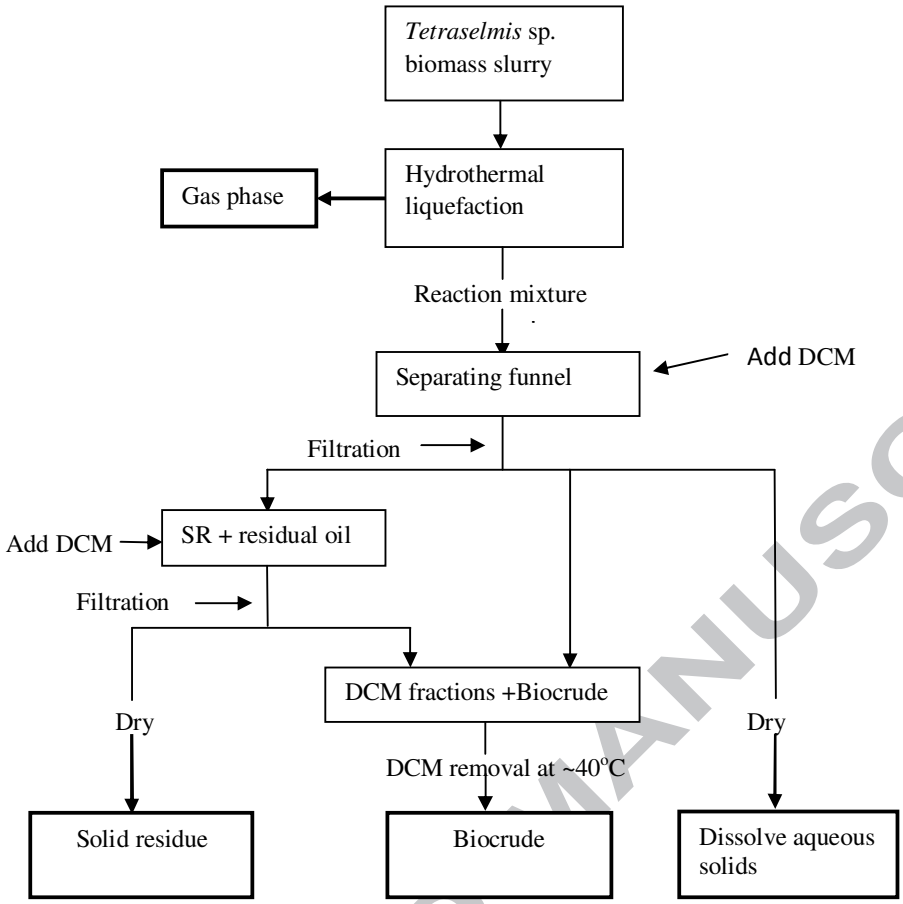


Figure 1: HTL of *Tetraselmis* sp., products recovery and separation procedure. DCM: dichloromethane, SR: solid residue.

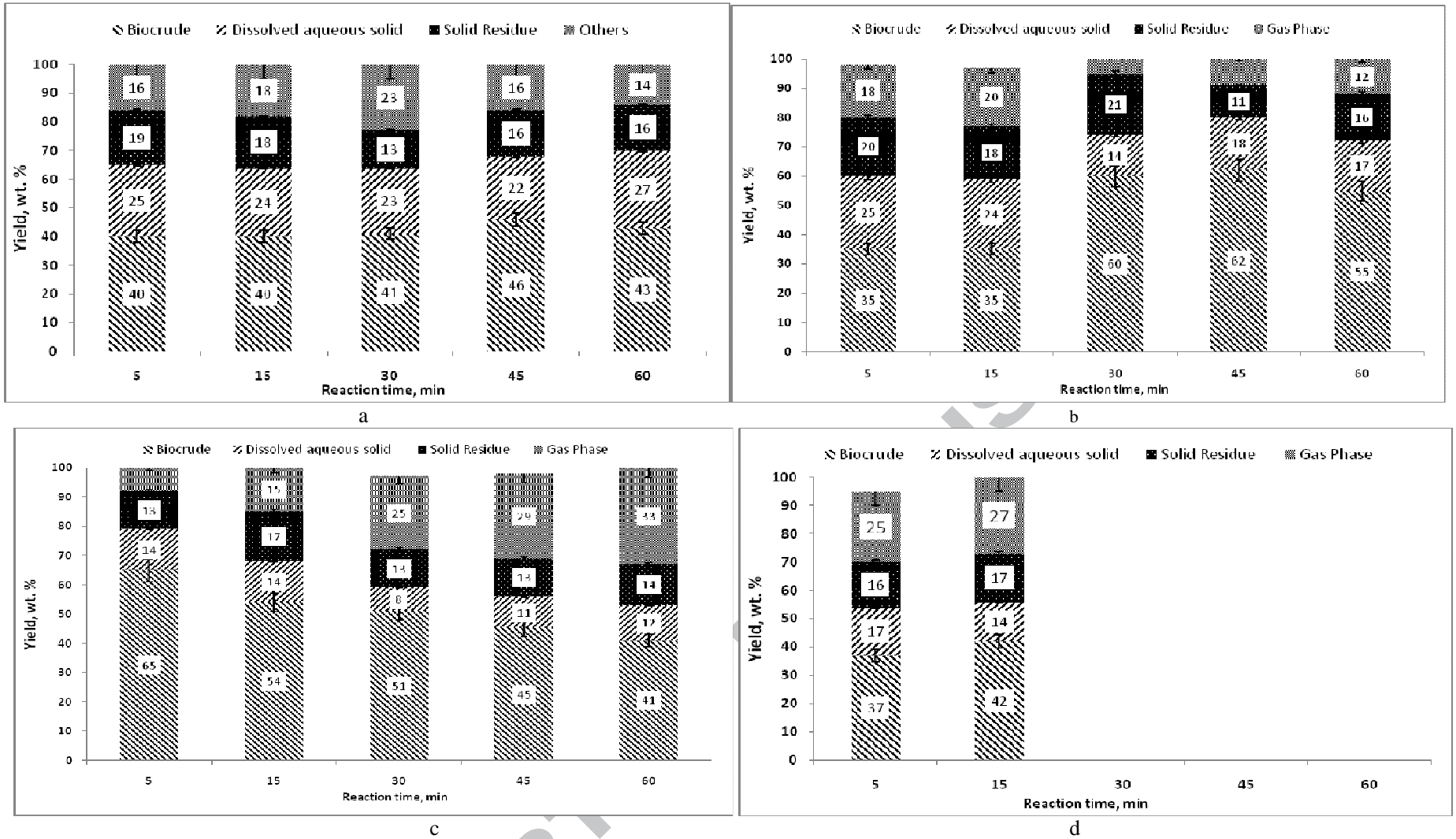


Figure 2: Mass yields in biocrude, aqueous phase, solid residues and gas phase from Tetraselmis **sp.** *at(a) 310°C (b) 330°C(c) 350°C and (d)370°C and holding time ranging from 5 to 60 min..*

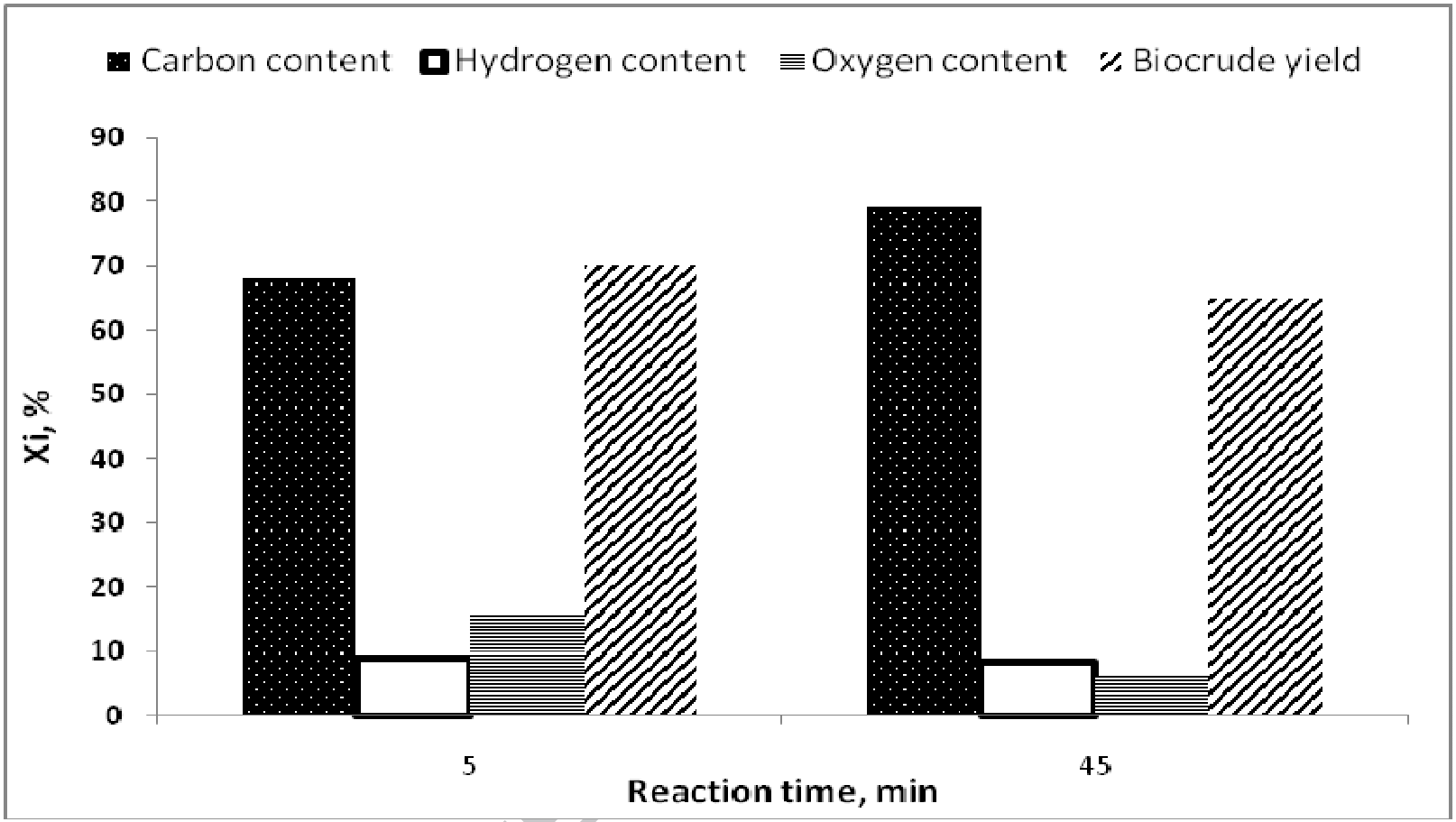


Figure 3: Comparison of the effect of reaction time on carbon and oxygen content in biocrude with its yield. X_i carbon, hydrogen, and oxygen content or biocrude yield in percentage.

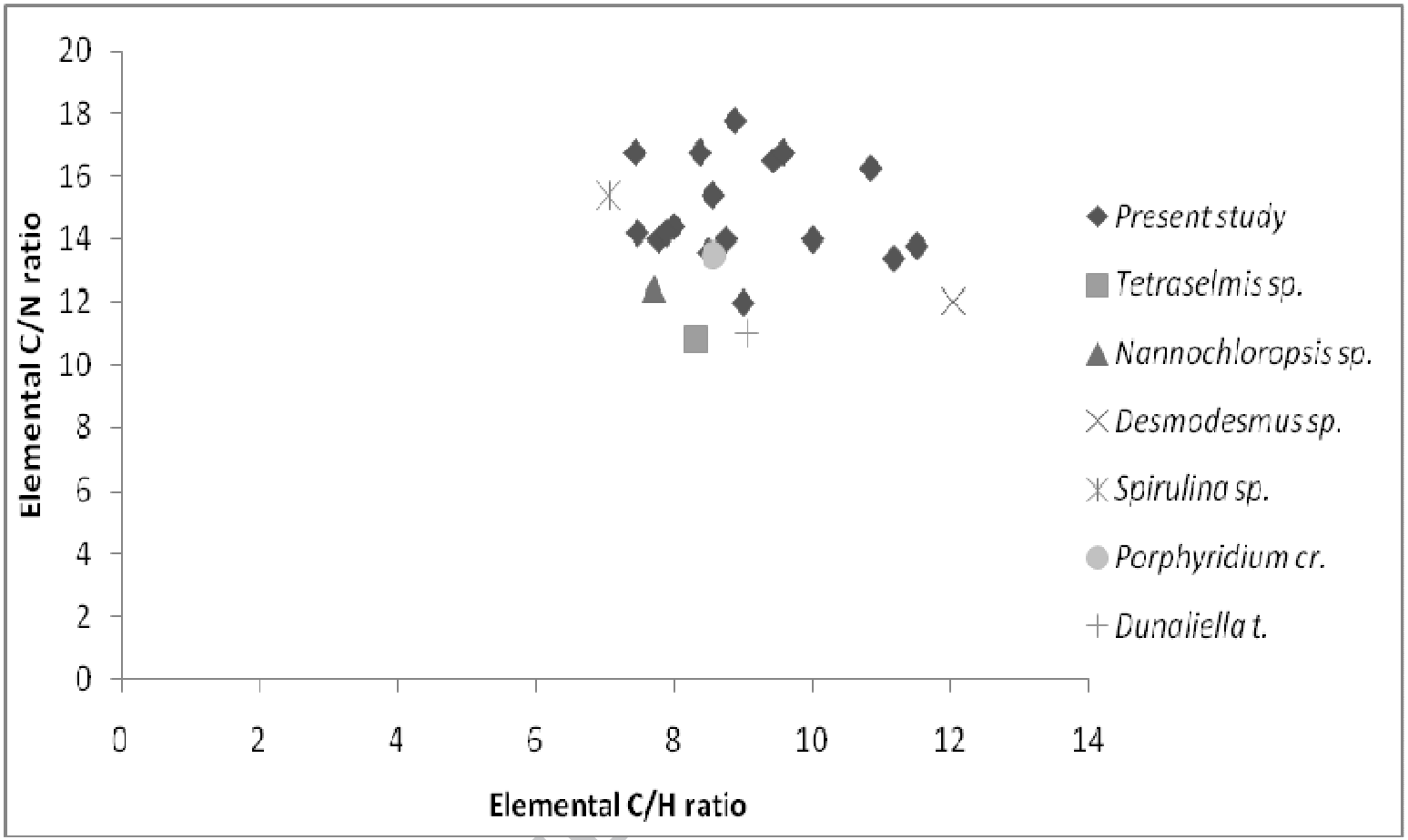


Figure 4a: Comparison of biocrude elemental Carbon-to-Nitrogen and Carbon-to-Hydrogen ratios obtained in the present study with other microalgal species.
Dunaliela tertiolecta (Minowa *et al*., 1995)*; Porphyridium cruentum (Biller and Ross, 2011); Spirulina* sp. (Biller *et al*., 2011); *Desmodesmus* sp.(Alba *et al*., 2012); *Nannochloropsis* spp

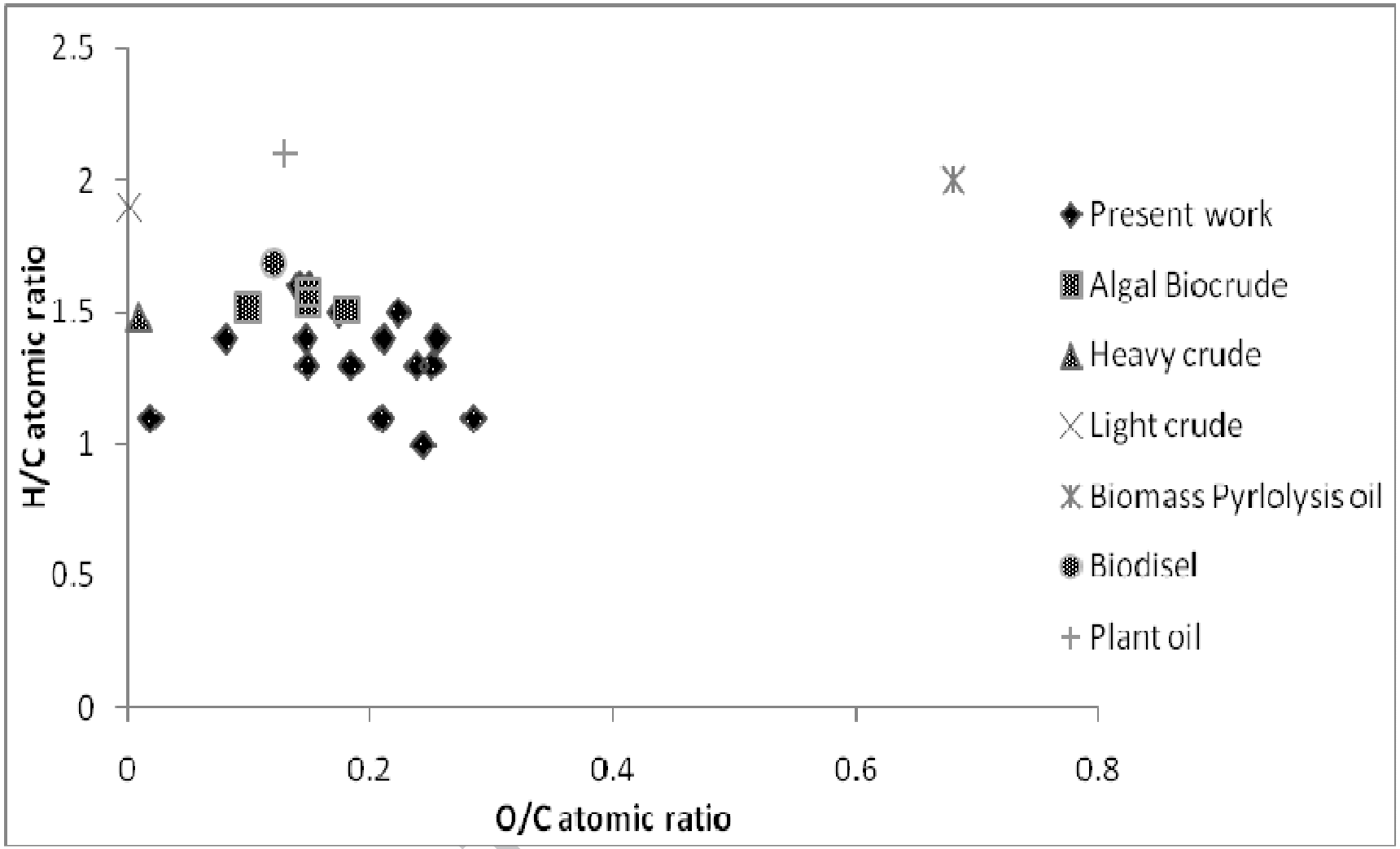
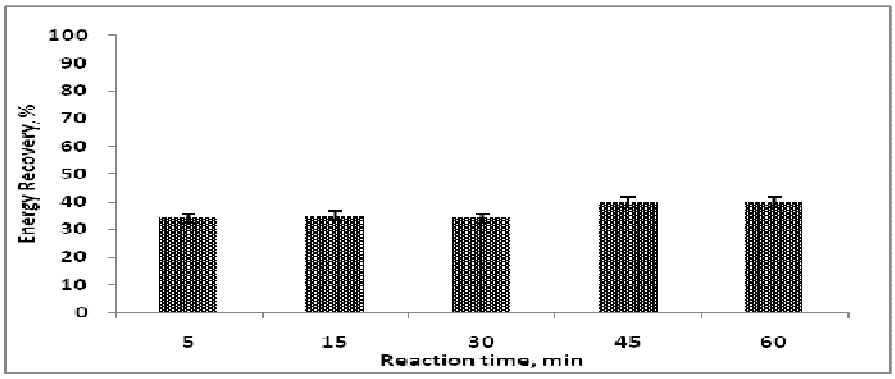
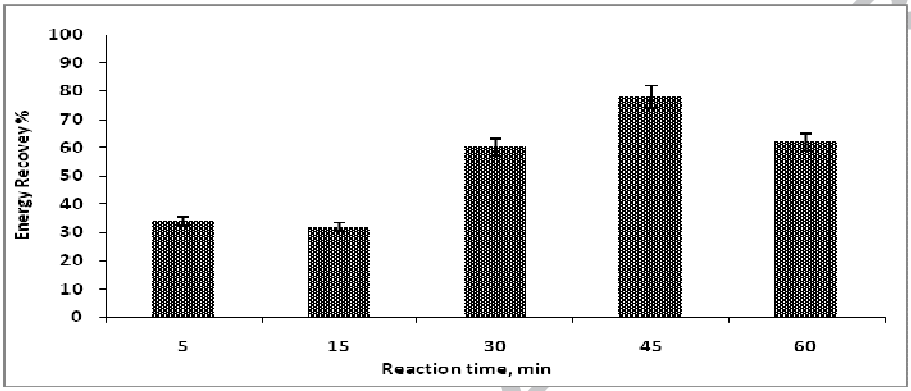


Figure 4b: van Krevelen diagram for different oils



a



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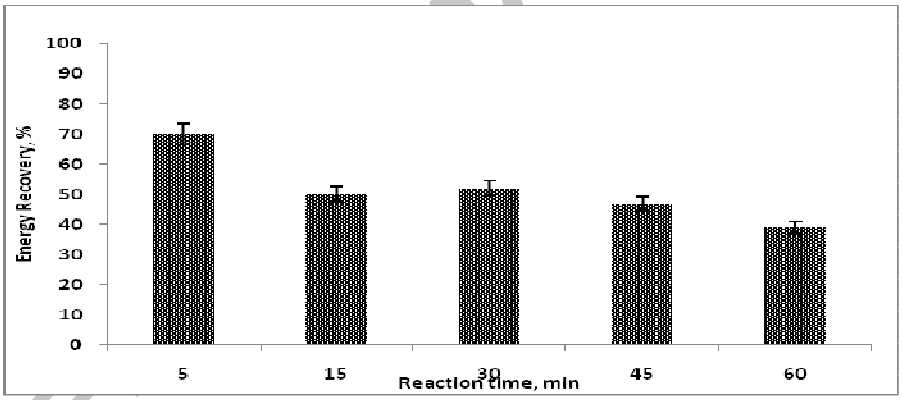

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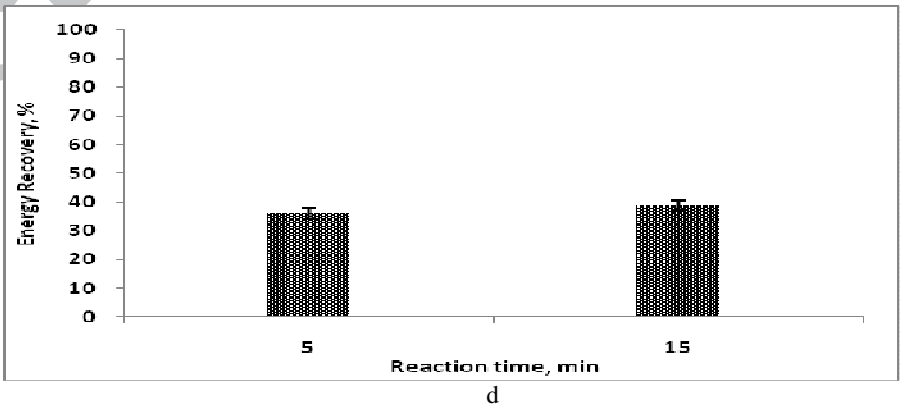

d

Figure 5: Effect of operating temperature and reaction time on energy recovery in HTL biocrude produced from Tetraselmis sp at *a: 310°C; b: 330°C; c: 350°C; d: 310°C, 5-60min.*

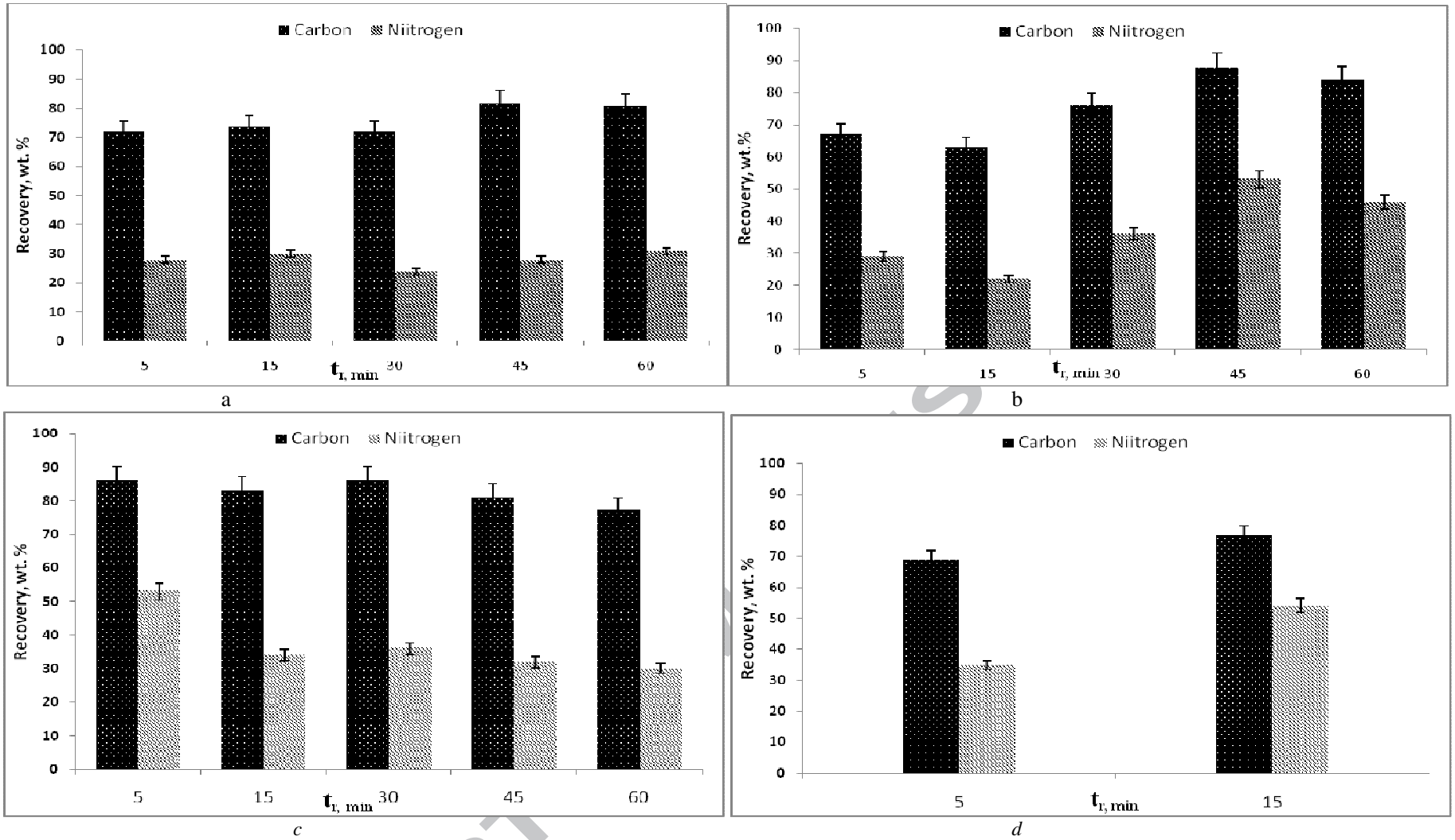

Figure 6:Effect of Temperature and reaction time on carbon and nitrogen recovery in HTL biocrude produced from Tetraselmis sp. (a): 310°C, (b) 330°C (c) 350°C and (d)370°C; tr: reaction time.

Strain	C	H	N	S	O	H/C ratio	HHV (MJ/Kg)[b]	Proteins	Carbohydrates	Lipids	Reference
			(%)						(%)		
Nannochloropsis oculata	57.8	8.0	8.6		25.7	1.66	26.8	57	8	32	Biller & Ross (2011)
Chlorella vulgaris	52.6	7.1	8.2	0.5	32.2	1.62	23.3	55	9	25	’’
Porphyridium cruentum	51.3	7.6	8.0		33.1	1.78	23.3	43	40	8	‘’
Spirulina sp.	55.7	6.8	11.2	0.8	26.4	1.46	24.6	65	20	5	‘’
Desmodesmus sp.	51.9	7.31	6.86		33.87	1.69	23.1	38-44	13-20	10-14	Alba *et al.*, 2012
Tetraselmis sp.	42.0	6.8	8.0	3.0	40.2[a]	1.94	19.2	58	22	14	Present study

Table 1: Elemental and biochemical composition of microalgae. a: obtained by difference, b: recalculated using the Chinnawala and Parikh equation, 2002

Microalgae	C %	H %	N%	S %	O[a] %	H/C	HHV (MJ/kg)[b]	Log R_o[c]	References
Tetraselmis sp.	71	9.5	5	0.6	14.0	1.6	35.0	5.21	Present study
Tetraselmis sp.	72.4	8.7	6.7	n.r	12.2	1.4	34.0	5.31	Barreiro *et al*., 2013
Nannochloropsis sp.	72.6	9.4	5.8	0.63	11.8	1.6	35.3	5.21	Faeth *et al*., 2013
Desmodesmus sp.	75.8	8.6	6.3	n.r	9.1	1.4	35.4	5.31	Alba *et al*., 2012
Chlorella vulgaris	75.4	10.7	4.9	<0.20	10.7	1.7	38.0	7.91	Ross *et al*., 2010
Spirullina sp.	73.3	9.2	7	n.r	10.4	1.7	37.0	7.91	Biller *et al*., 2010
Porphyridium cr.	72.0	8.5	5.4	0.30	13.3	1.4	34.0	7.91	Biller and Ross, 2011
Petro crude[US & SA]	82.5-88.9	10.4-14.2	0.3	0.2-5.7	0.8	1.4-1.9	40-44		Speight, 1999

Table2: Properties of biocrude obtained at optimum conditions from hydrothermal liquefaction of different microalgae
US & SA: United States and South America; a: by difference, b: recalculated based on reported CHNSO data, n.r: not reported, c: calculated based on reported operating conditions

Highlights:

*Maximum biocrude yield of ~65wt% was achieved

*The maximum yield and quality of the biocrude are independent variables.

*High biocrude yield was favoured by short reaction time.

*Biocrude quality was favoured by long reaction time.