*Journal of Experimental Research*

*December 2019, Vol 7 No 4*

***Email: editorinchief.erjournal@gmail.com***
***editorialsecretary.erjournal@gmail.com***

***Received:*** *29th February, 2020*
***Accepted for Publication:*** *22nd Oct., 2019*

# Co-liquefaction of Hypersaline *tetraselmis* sp. Microalga and Cow Manure for Biocrude Production and as Means for Solid Waste Treatment

**Eboibi BE**

Department of Chemical and Petroleum Engineering,
Delta State University, Oleh Campus, Delta State, Nigeria.

***Author for Correspondence:** blessingeboibi@gmail.com

**ABSTRACT**

This paper investigated hydrothermal coliquefaction of the microalga *Tetraselmis* sp. and cow manure at different mix ratios and the characterisation of produced biocrude. The carbon and nitrogen balances across the reactor and energy recovery were also elucidated. The study was conducted using a 1L batch reactor at 300°C and 350°C at constant reaction time of 10min using ~16w/v% solids loading. The results showed that irrespective of reaction temperature, there were substantial influence on yield and properties of biocrude. Importantly, there were up to 60% reductions in nitrogen content of biocrude, which could be due to synergistic effect from interactions of feedstock molecules during liquefaction. These findings suggest blending of *Tetraselmis* sp. and cow manure could improve biocrude quality, while simultaneously treating waste.

**Key words:** Hypersaline, Microalgae, hydrothermal, co-liquefaction. Cow manure

## INTRODUCTION

Hydrothermal liquefaction (HTL) of microalgae biomass to biocrude is a promising process for biofuel production (Huang et al. 2019). Importantly, HTL avoids use of energy-intensive in drying of feedstock, as required in other thermochemical methods such as pyrolysis (Eboibi et al., 2014). However, due to current challenge in production cost of microalga, its treatment in a single-stream could negatively impact future commercialization of the process (Brilman et al. 2017; Giaconi et al. 2017). In addition to improving yield and quality of biocrude, presence of heteroatoms such as nitrogenous and oxygenated compounds in biocrude have remained an issue. Thereby inducing replacing conventional fossil crude in nearest future. Therefore, more scientific research on suitable feedstock selection as co-feeds alongside algae liquefaction and means of reducing heteroatom's is necessary.

Due to potential synergistic impact on yield and quality of biocrude, low logistics costs for feedstock collection and transportation, it is believed that co-liquefaction of different organic biomass in a single-stream is advantageous compared to liquefaction of individual feedstock (Yang et al. 2019). Co-liquefaction of algae and solid organic waste, in this instance animal manure has been suggested to enhance economic sustainability of HTL-algae-biofuel (Giaconi et al. 2017; Lam et al. 2019).Importantly, co-liquefaction has the potential to enhance yield and properties of biocrude through adjusting the biochemical composition of feedstock mixtures (Yang et al. 2017). This would not only improve the yield and quality of biocrude (Xu et al. 2019; Yuan et al. 2019) but also addresses issues relating to handling and disposal of manure (Eboibi et al. 2015). As manure management has been a concern to agro industries (Usapein and Chavalparit, 2017).

Approximately 921m metric tons of wet manure was produced from 77.6m animal units of cattle in the United States (USDA, 2007). Although, manure is applicable on fields as traditional/or suitable methods for manure management, increases in urbanization and strict environmental policies seems to make this option limited (Saba et al. 2018). As this option may lead to increase in greenhouse gas and particulate emissions (Baldé et al. 2016). Manure contains essential nutrient (such as phosphorus and nitrogen), its improper management could lead to run offs, affecting water quality (such as eutrophication), public health and surrounding ecosystems (Cantrell et al. 2017; Sharpley, 1981).Therefore, co-liquefaction of microalga and animal manure has broad application potentials; provide resource recovery, reduction in carbon footprint, and in waste treatment to mitigate environmental pollution (Huang et al. 2019; Wu et al. 2017).

A review of the scientific literature showed limited study on co-liquefaction of microalgae and cow manure, though there has been a reported study on individual liquefaction of cow manure and algae. Few studies have evaluated the co-liquefaction of microalgae with other organic biomass as co-feedstock. These include co-liquefaction of microalgae and; microalgae (Jin et al. 2013); agricultural waste (Chen et al. 2019; Wang et al. 2019); sewage sludge (Xu et al. 2019); polypropylene (Wu et al. 2017) and the use of mixed algae strains (Dandamudi et al. 2019; Hietala et al. 2019) and model compounds (Feng et al. 2019; Zhang et al. 2016). The results of these studies are contradictory, generally; the effects are either synergistic when combined feeds yields are higher than individual feed (Gai et al. 2015; Xu et al. 2019) or antagonistic (Brilman et al. 2017; Chen et al. 2019) when reverse is the case. Also, the elemental carbon and nitrogen content distributions are inconsistent (Yang et al. 2019). This development therefore suggests more scientific research investigation.

In addition, previous studies used pulverised algae and combined feeds for their experimental studies. Such practices are may be acceptable at laboratory scale unlike in commercial scale. The properties and structure of the feeds may be altered prior to liquefaction, which may affect output and quality. The use of freshly harvested microalgae seems to present real-life scenario unlike pulverised algae. Therefore the main aim of present study is to fill the knowledge gap.

## MATERIALS AND METHOD

### Materials:

Freshly harvested hypersaline *Tetraselmis* sp. alga (TA), and cow manure (M) was used in the present study. TM was grown and cultivated in outdoor open raceway ponds. Its cultivation and harvesting has been reported elsewhere (Isdepsky1and Borowitzka1, 2019; Fon-Sing et al. 2014). Cow manure (CM) was obtained from a local farm at Thandallam, Chennai, India.

### Analytical Method:

HTL experiment were conducted using a 1L batch high-pressure reactor made of Inconel at reaction temperature of 300$^{o}$C and 350$^{o}$C at constant reaction time of 15min, using biomass feedstock containing ~20%w/w solids. Typically, for individual run, 500g of either TA or CM was loaded in the reactor. For co-liquefaction studies, TA and CM were mixed in ratio 04:01, 03:02, 01:01, 02:03 and 01:04. The production and separation procedures were carried out in accordance with previous reports (Eboibi et al. 2014; Wang et al. 2019). For repeatability, each experimental run was carried out in triplicate, and the average result reported.

The elemental and biochemical composition of TA and CM, including that of previous studies are shown in Table 1. A Variol III Elemental Analyser System GmbH was used to determine the weight percentages ofcarbon (C), hydrogen (H), nitrogen (N) and sulfur (S) following ASTM D-5291 method. The oxygen content was estimated by subtraction from the combined mass of CHN and S.

After product separation, the primary product biocrude yield was determined in wt% on an ash free dry wt. basis. The solid residue and dissolved aqueous solids were estimated in weight percent by relating their mass yield to that of feed loaded in the reactor (Theegala and Midgett, 2012). The gas phase yield was estimated by difference by subtracting the combined mass yield of biocrude, solid residue and dissolved aqueous solids from unity.

Based on the data from the elemental analysis, the higher heating value (HHV) was estimated using Eq. (1), proposed by Chinnawala and Parikh, (2002), while the molar atomic ratios of H/C, O/C N/C were estimated in accordance to previous reports (Alba et al. 2012; Eboibi et al. 2019).

An Official Publication of Enugu State University of Science & Technology ISSN: (Print) 2315-9650 ISSN: (Online) 2502-0524

$$HHV\ \frac{MJ}{kg} = 0.3491C + 1.1783H + 0.1005S - 0.01340 - 0.0151N - 0.0211A \quad (1)$$

where C represents carbon, H hydrogen, S sulfur, O oxygen, N nitrogen and A ash, on dry basis.
The amount of energy recovered (ER. %) was estimated using Eq. (5).

$$ER = \frac{HHV\frac{MJ}{kg}\text{of biocrude * weight of product (g)}}{HHV\frac{MJ}{kg}\text{of feed * weight of feed (g)}}\ X\ 100\% \quad (5)$$

It should be noted that the external work applied for heating the reactor was not considered in Eq. (2), but by relating the HHVs and mass of biocrude to that of initial biomass load fed to the reactor (Biller and Ross, 2011). The C and N recovery (%) was calculated by using the elemental mass balances across the reactor (Eboibi et al. 2014).

## RESULTS AND DISCUSSION

**Mass yields:**

The yields obtained from HTL of individual and combined feed is presented in Figure 1. The treatment at 300°C (Fig. 1a), with 10min reaction time using 20% solid loading led to 32 to 42, 15 to 24, 12 to 15 and 21-34wt% biocrude, solid residue, dissolved aqueous solids and gas phase yields, respectively. An increase in reaction temperature from 300°C to 350°C (Fig. 1b), led to substantial increase in biocrude yield when compared to that obtained at 300°C. For individual treatment, biocrude from TA increased from 42wt% to 58wt%, and from 32wt% to 42wt% for CM. In contrast, the solid residue reduced from 24wt% to 16wt% and 14wt% to 10wt% for TA and CM, respectively. This variation in yields with respect to reaction temperature could be attributed to promotion of decomposition of reactants including polymerisation of intermediates (Wu et al., 2017).Similar trend was found for the combined feeds; as there were general reduction in solid residue from 24wt% to 13wt%, and 15wt% to 11wt% for dissolved aqueous solids. However the gas phase yields increased numerically at all conditions. The relative increase in biocrude yields from co-liquefaction could be due algae containing alkali salts. Alkali salt is known to enhance biomass macromolecules degradation into biocrude. Hence, blending of TA with biomass materials with or without alkali metals would improve biocrude yields.

The low biocrude yield and relatively high solid residue yields obtained at 300°C compared to at 350°C could be due to insufficient conversion of the feedstock. It is possible some bonds were unbroken due to perhaps inadequate reaction temperature and or reaction time. In addition, the biochemical composition (protein, carbohydrate and lipids) of the feedstocks could have influenced the yields. Microalgae containing higher lipids potentially produce higher biocrude and lower solid residue yields unlike algae containing higher carbohydrates. Consequently, coliquefaction of algae with high lipids and with other biomass of higher carbohydrate would lead to lower biocrude yield (when compared to HTL of individual algae) and higher yield (when compared with HTL of the other biomass with higher carbohydrate component). Consequently, coliquefaction of algae with high lipids and with other biomass of higher carbohydrate would lead to lower biocrude yield (when compared to HTL of individual algae) and higher yield (when compared with HTL of the other biomass with higher carbohydrate component). Carbohydrate has been reported to contribute little to the overall bio-crude yield, and at the sometime neutralizes the negative effect of protein and enhance the performance of HTL by the Maillard reaction at an optimal ratio (Zhang et al. 2016).

| Feedstock | Biochemical composition | | | | | | Elemental composition | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Carbohydrate | Proteins | Lipids | Cellulose | Hemicellulose | lignin | C | H | N | S | O | H/C | HHV | |
| *Tetraselmis* sp.[a] | 22 | 58 | 14 | - | - | - | 42 | 6.8 | 8 | 3 | 40.2 | 1.94 | 18.3 | Present study |
| Cow manure[b] | 58 | 11 | 15 | - | - | - | 35.6 | 6.5 | 2.1 | 0.6 | 55.2 | 2.19 | 14.3 | |
| *Spirulina plantensis* | 23.9 | 70.3 | 5.8 | - | - | - | 46.9 | 6.9 | 10.7 | NR | 35.5 | 1.77 | 18.7 | Feng *et al.*, (2019) |
| α-Cellulose | - | - | - | 99.6 | - | - | 44.4 | 6.1 | 0 | NR | 40.3 | 1.67 | 16.0 | |
| *Nannochloropsis* sp. | 12.4 | 36.4 | 19.0 | - | - | - | 49.2 | 7.2 | 6.2 | NR | 36.3 | NR | 20.5 | Zhang *et al*., (2016) |
| *Spirulina plantensis* | 10.8 | 48.5 | 5.8 | - | - | - | 45.6 | 6.6 | 10. | NR | 36.7 | NR | 18.4 | |
| *Cyanidioschyson merolae* | 37.8 | 47.8 | 4.3 | - | - | - | 48.1 | 5.1 | 9.9 | 1.2 | 35.5 | NR | 18.1 | Dandamudi *et al.*, (2017) |
| *Galdiera sulphuraria* | 42.2 | 45.1 | 3.2 | - | - | - | 42.4 | 3.9 | 9.4 | 1.3 | 42.9 | NR | 16.4 | |
| *Spirulina* sp. | 29.3 | 26.8 | 11.0 | - | - | - | 34.5 | 5.1 | 3.4 | NR | 24.2 | NR | NR | Jin *et al*., (2013) |
| *Entermorpha prolifera* | 18.2 | 23.8 | 5.0 | - | - | - | 28.0 | 4.5 | 3.8 | NR | 10.7 | NR | NR | |
| *Chlorella pyrenoidosa* | 20.6 | 57.6 | 10.6 | - | - | - | 47.2 | 8.3 | 8.9 | 0.3 | NR | NR | 22.6 | Chen *et al*., (2019) |
| Sweet potato residue | 20.7 | 1.7 | NR | 25.9 | 12.7 | 34.5 | 47.2 | 7.2 | 0.2 | NR | 40.5 | NR | 20.4 | |

[a]Eboibi *et al*., (2014)
[b]Eboibi *et al*., (2015)

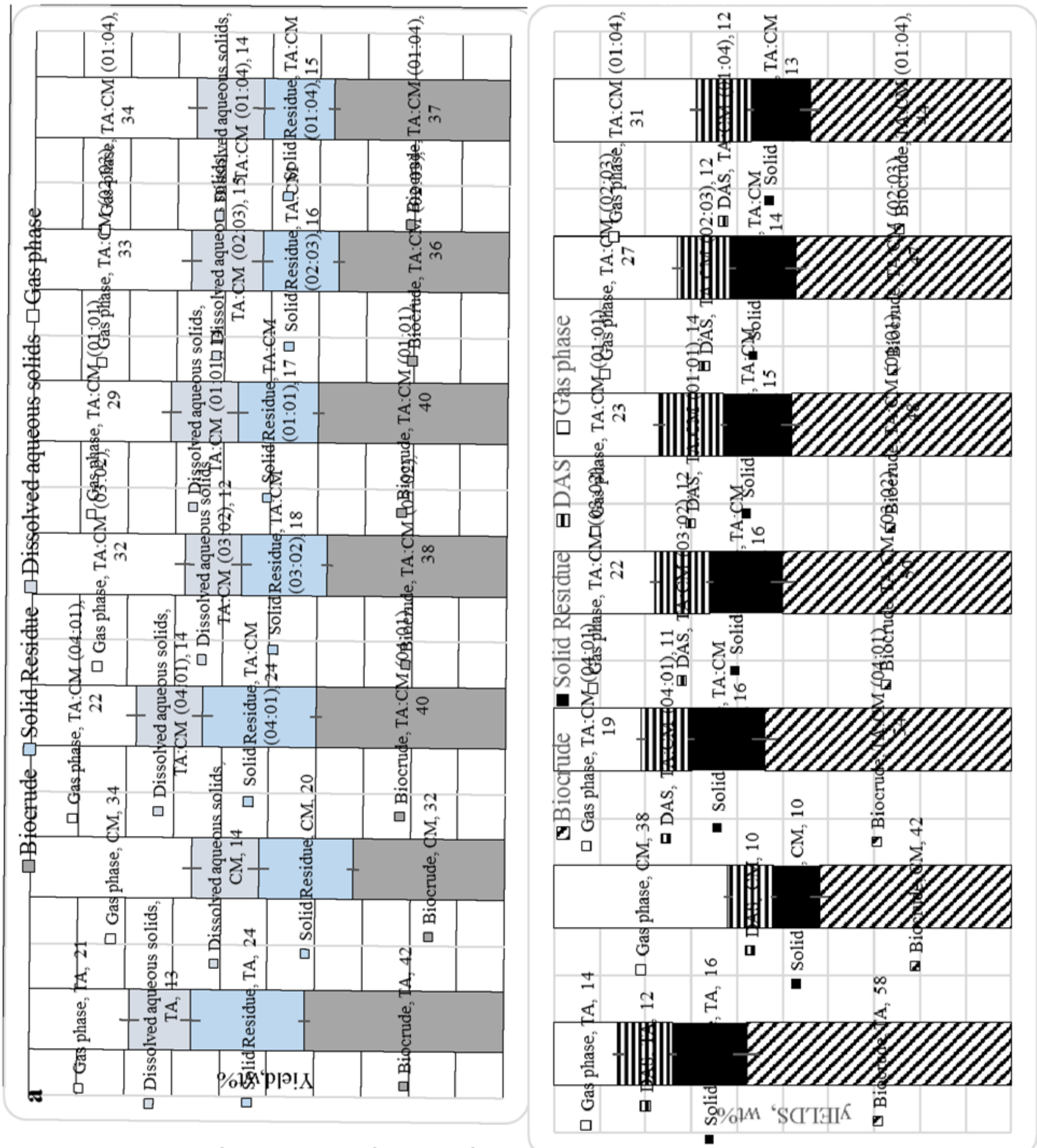


**Fig. 1: Mass yields from co-liquefaction of *Tetraselmis* sp. alga (TA) and cow manure (CM) at 10min constant reaction time. A: 300°C, B: 350°C.**

This therefore suggests that for optimal biocrude yield, the composition of feedstocks needs to be varied, in order to achieve a suitable feedstock mix ratio. This study has shown that blending different feedstock have substantial positive effects on distribution and potentially enhances the biocrude yield. This is advantageous of using all kinds of available biomass wastes (Yuan et al. 2019) for biofuel production. Although, there are limited data on the synergistic effect (SE) on co-liquefaction of algae and cow manure, a SE of -4.2 to ~7 was found and which is within the range of previous related studies (shown in Table 2). Yang et al. (2019) reported that co-liquefaction of microalgae with other biomass types would most likely lead to SE of about 2.2 to 8.7wt% on biocrude yield.

**Table 2: Summary of some previous research investigation on co-liquefaction of algae and other biomass feedstocks**

| Feedstock | Optimum reaction condition T ($^oC$), T(min), SL(wt.%) | IY biocrude yield, wt.% | Optimal mixed ratio | Mixed feedstock biocrude yield, wt.% | | | Biocrude properties Carbon | | Nitrogen | | ER,% | | HHV(MJ/kg) | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Expt. | Cal | SE | IY | Col | IY | CoL | IY | CoL | IY | CoL | |
| *Spirulina plantensis* | 300, 30 | 35.6 | 2:1 | 40.3 | 24 | +16 | NR | NR | NR | NR | 50 | 82 | 33 | 30 | Feng *et al*., (2019) |
| α-Cellulose | | 14.0 | | | | | NR | | NR | | 28 | | 28 | | |
| *Nannochloropsis* sp. | 280, 60 | 37.9 | 1:1 | 28.96 | 33.4 | -4.4 | 72.7 | 73.2 | 4.6 | 6.7 | 66.4 | 54.4 | 35.9 | 36.5 | Zhang *et al*., (2016) |
| *Spirulina plantensis* | | 29.2 | | | | | 70.9 | | 7.2 | | 54.2 | | 34.1 | | |
| *Cyanidioschyson merolae* | 300$^o$C, 30, 20 | 18.9 | 80:20 | 25.5 | NR | NR | 78.0 | 74.4 | 3.7 | 7.6 | NR | NR | 33.8 | 35.2 | Dandamudi *et al*., (2017 |
| *Galdiera sulphuraria* | | 14.0 | | | | | 76.6 | | 6.2 | | NR | | 36.4 | | |
| Mixed-culture algal strain | 300, 60, 25 | 27 | 1:3 25:75 | 35.7 | NR | NR | 59.4 | 35.7 | 2.5 | 2.5 | 52 | 37 | 25.8 | 17.8 | Chen *et al*., (2014) |
| Swine manure | | 40 | | | | | 76.6 | | 3.5 | | 83 | | 38.8 | | |
| *Spirulina* sp. | 340, 40, | 24.7 | 1:1 | 21.6 | 24.8 | +3.2 | 74.2 | 74.3 | 4.1 | 5.3 ^ | 56 | 54 | 35.5 | 35.3 | Jin *et al*., (2013) |
| *Entermorpha pro.* | | 14.6 | | | | | 74.1 | | 4.5 | | 35.2 | | 34.9 | | |
| *Chlorella pyre.* | 300, 60 | 40.5 | 4:1 | 40.4 | 38.1 | -2.3 | 73.5 | 75.2 | 7.6 | 7.1 | NR | 67 | 35.3 | 35.4 | Chen *et al*., (2019) |
| Sweet potato waste | | 37 | | | | | 70.8 | | 0.3 | | NR | | 29.6 | | |
| *Chlorella sp.* | 340, 30, ~10 | ~22 | 1:1 | 26.8 | 22.1 | +4.7 | 72.0 | 70.8 | 6.5 | 6.5 | 41.6 | 57.8 | 34.1 | 33.4 | Xu *et al*., (2019) |
| Sewage sludge | | ~24 | | | | | 71.8 | | 6.0 | | 52.7 | | 34.2 | | |
| *Dunaliella tertiolecta* | 340, 40, 10* | 28 | 2:8 | 27.42 | 30.7 | +3.3 | NR | NR | NR | NR | NR | NR | NR | NR | Wu *et al*., (2017) |
| Polypropylene | | 1.82 | | | | | NR | | NR | | NR | | NR | | |
| *Desmodesmus sp.* | 350, 10, 10 | 53 | 1:1 | 29 | 36 | -7 | NR | NR | 6 | 4.2 | 71 | ~43 | 33 | 32 | Brilman *et al*., (2017) |
| Pine wood | | 24 | | | | | NR | | 1 | | 40 | | 30 | | |

As shown in Table 2, Feng et al. (2019) investigated co-liquefaction of a low-lipid microalgae *Spirulina* sp. and α-Cellulose at reaction temperature of 300°C at 30min reaction time. At an optimal 2:1 mix ratio, SE of ~16wt% on biocrude yield was obtained. Operating at a reaction temperature of 340°C, 30min reaction time and using 10w/v% solids content, Xu et al. (2019) reported SE of 4.7wt% at 1:1 mix ratio. Similarly, Jin et al. (2013) reported SE of 3.2wt% on biocrude yield from the co-liquefaction of *Spirulina* sp. and *Entermorpha prolifera* at 340°C, 40min at ratio 1:1. In contrast to these SE, some investigations (Brilman et al. 2017 and Zhang et al. 2016) have shown negative effect on biocrude yields following co-liquefaction (Table 2). The reported AE were majorly due to the biochemical composition of the feedstock. For example Chen et al. (2019) and Dandamudi et al. (2017) used had feedstock with high carbohydrate and/or low lipids for their studies (Table 1), hence the low biocrude yield (shown in Table 2). Nevertheless, most reported studies have shown improved quality of the biocrude obtained from co-liquefaction when compared to individual feedstock, which will be discuss in next session. Importantly, more scientific research investigation is required to understand the underlying mechanism for the observed co-liquefaction effects.

**Elemental composition of biocrude:**

The elemental carbon, hydrogen, nitrogen, sulfur and oxygen content for biocrudes obtained at different mix ratio and reaction temperature is shown in Fig. 2. As illustrated in Fig. 2, the reaction temperature and co-liquefaction of *Tetraselmis* algae (TA) with cow manure (CM) had substantial impact on the elemental content of the biocrudes. Generally, operating at 350°C (high temperature (HT)) led to an increase fractionation of elemental carbon and hydrogen in resultant biocrudes when compared to that at 300°C (low reaction temperature (LT)).Co-liquefaction of TA and CM was found to have substantial effects on the elemental distribution in biocrudes, irrespective of reaction temperature.

Furthermore, higher mass of TA in the feedstock mix led to numerical increase in carbon and hydrogen content in biocrude when compared to individual CM. Leading to enhanced energy density of biocrude, thus improving biocrude quality. For example, irrespective of reaction temperature, biocrude obtained from mix ratio 04:01 (TA:CM) led to 72w/w% of carbon compared to 68w/w% from 01:04 (TA:CM) mix ratio. However, for individual liquefaction of TA, co-liquefaction led to relatively low carbon and hydrogen content and high oxygen content in resultant biocrude. Suggesting that co-liquefaction promotes deoxygenation and decarboxylation reactions. Chen et al. (2019) reported substantial reductions in oxygen and improved carbon recovery in produced biocrude.

More CM in the feedstock mix was found to substantially reduced N- content in biocrude when compared to individual liquefaction of TA. As shown in Fig. 2, an increase in CM mass in the blend CM and TA, the lower the nitrogen content of biocrude. This led to about 50% reduction in N-content in resultant biocrude. Suggesting combining biomass feedstock of lower nitrogen with algae would reduce the recovery of nitrogen, thus reduction in $NO_x$ emissions upon combustion. Wang et al. (2019) reported similar trend, as more dosage of sweet potato residue (SPR) biomass in blend of *Chlorella pyrenoidosa*and SPR led to reduction of N-content from 6.77w/w% to 3.20w/w%. They conducted their co-liquefaction studies at 300°C and 60min reaction temperature and reaction time, respectively.

Similarly, Chen et al. (2019) investigated the co-liquefaction of *Chlorella pyrenoidosa* and potato biomass at 300°C and 60min and reported substantial reduction in N-content of biocrude from7.6w/w% to 4.4%.Algae biomass are generally known to contain high nitrogen, which is one of the important challenges of HTL-algae biofuel (Eboibi et al., 2015, Tang et al. 2020). Therefore, this approach could help to address this issue. Furthermore, based on the data presented in Figure 2, the optimal mix ratio of TA:CM (01:01) was found more suitable for more recovery of element in biocrude, except oxygen which was found for 04:01. However, since one of the objectives was also to improve biocrude yield, via co-liquefaction, a TA CM ratio of 03:02 was found more suitable for higher biocrude yield at 350°C.

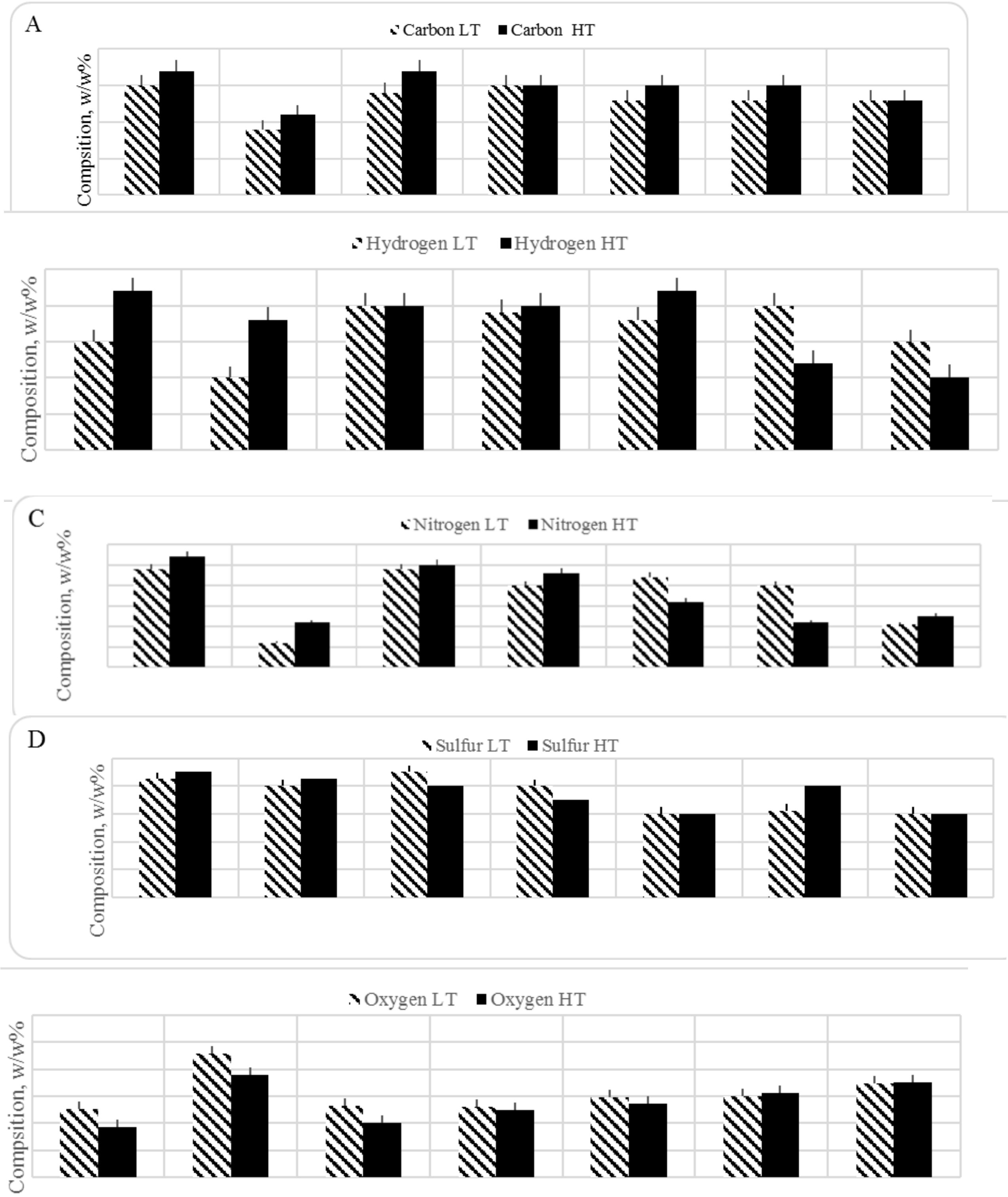


**Fig. 2: Elemental composition of biocrude obtained at different feedstock mixing ratio**

A: Carbon content B: Hydrogen content. C: Nitrogen content. D: Sulfur content. E: Oxygen content. LT: low reaction temperature (300°C). HT: high reaction temperature (350°C).

**Effects of co-liquefaction on molar atomic ratios**

The H/C, O/C and N/C molar atomic ratios of biocrudes obtained from individual TA and CM and at different mixing ratios are presented in Fig. 4 (the Van Krevelen diagram). As illustrated in Fig. 3A, the biocrude derived from *Tetraselmis* sp. (TA) had a better H/C and O/C ratio when compared to those obtained from cow manure and the mix feeds, however, substantially lower than that of petrocrude, with H/C close to 2 and O/C close to unity. Also, the biocrudes obtained from different mix ratios in have improved O/C atomic ratios and in most cases relatively enhanced H/C ratios. The improved O/C and H/C atomic ratios could be majorly due to dehydration and deoxygenation reactions. This finding suggests that co-liquefaction of microalgae and other organic biomass such as cow manure could improve the quality of biocrude.

The H/C with N/C atomic ratio of 3B) biocrudes (Fig. 3B) further shows the advantages of co-liquefaction. Due to CM having lower nitrogen content (Table 1), biocrude obtained from CM has a better H/C and N/C ratio when compared to that of other biocrudes from TA and mix feeds. Interestingly, biocrude H/C and N/C atomic ratios of mixed feedstocks were found to be lower when compared with that of individual TA. Apparently, this finding has shown the importance of blending feedstock of lower N-content with algae, as it potentially reduces the nitrogenous compounds in produced biocrude. Such practices are seen to improve biocrude quality. It could be concluded that addition of feedstock with no/or low nitrogen content, in this case CM with microalgae enhances Mannich reaction, hence the reduction in N-content of produced biocrudes.

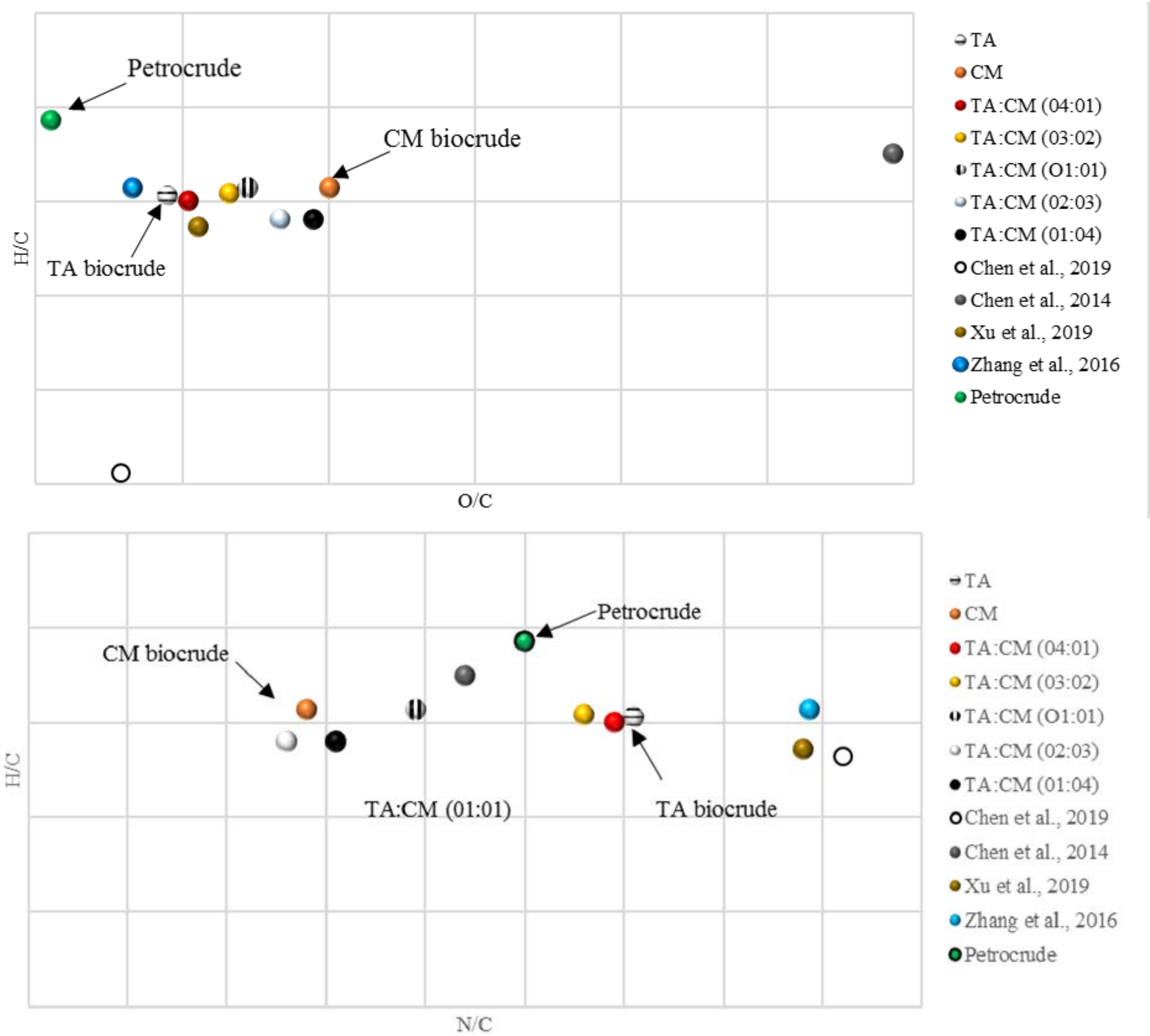


**Fig.3: Van Krevelen diagram showing atomic ratios after co-liquefaction at 350°C.**

A: Hydrogen-to-carbon (H/C) against oxygen-to-carbon ratio (O/C). B: Hydrogen-to-carbon ratio against nitrogen-to-carbon (N/C) ratio.

**Carbon and nitrogen recovery:**

One of the factors to determine quality of biocrude is the amount of carbon and nitrogen recovered in the biocrude. As more amount of carbon recovery (CR) and lower nitrogen recovery (NR) in the biocrude, the better the quality. Higher amount of CR the denser is the energy and performance. Also, lower amount of N-content in biocrude the better the quality. However, biocrude denitri?cation has remains an important challenge in its quality (Chen *etal.* 2019). Consequently, scientific research effort towards nitrogen reduction in biocrude would of interest. The amount of CR and NR in biocrudes obtained from mixed and individual feedstock is illustrated in Fig. 4.

Based on the data presented in Fig 4A, more carbon were recovered in biocrude at higher reaction temperature (HT (350°C)) when compared to that at lower reaction temperature (LT (300°C)).CR were found to correspond with the yield in biocrude, as an increase biocrude, the more CR in biocrude. Similarly, NR was found to follow similar trend for CR with respect to reaction temperatures. This finding seems to be in agreement with previous studies on decomposition of nitrogen with respect to reaction temperature. Decomposition of nitrogen in biocrude following HTL has been shown to increase with an increase in reaction temperature (Eboibi *et al.*, 2014)

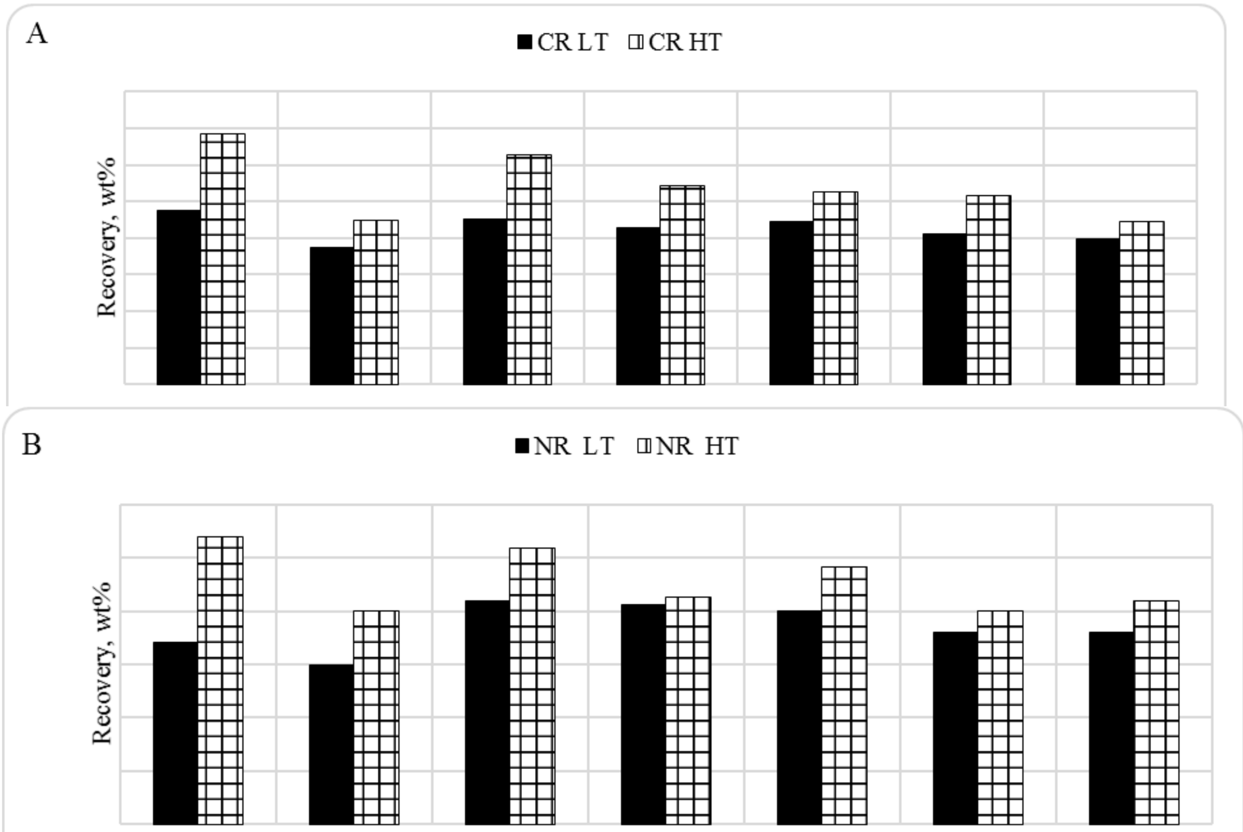


**Fig. 4: Carbon and nitrogen recovery from co-liquefaction of TA, CM and at different ratios**

CR: carbon recovery. NR: nitrogen recovery. LT: low temperature (300°C). HT: high temperature (350°C). TA: *Tertraselmis* sp. algae. CM: Cow manure

As shown in Fig.4, biocrude obtained from mixed feedstock had relative lower amount of CR and NR in biocrude, especially when compared to TA biocrude. However, when compared with CM biocrude, CR and NR from mixed feedstock were higher. Suggesting quality and yield of biocrude derived co-liquefaction is dependent on combined feedstock. In this present study, NR reduces thus improving the quality of biocrude as the proportion of CM increased in the mixed feedstock. However, CR in biocrude relatively decreases, reducing the energy density of the biocrude. Nevertheless, using TA and CM as mixed feedstock for HTL-biocrude seems more advantageous, in terms of lower NR, potentially reduction in pollution and a viable option for waste management.

**Energy density: Energy recovery and HHV**

Another important factor in terms of biocrude quality and HTL reaction efficiency is the amount of energy recovery (ER) and HHV (Biller and Ross, 2011, Wang et al., 2019).The ER and HHV of biocrudes are presented in Fig. 5. As illustrated in Fig. 5 both reaction temperature and co-liquefaction had substantial effects on the energy recovery and HHVs of the biocrudes. For individual feedstock, about 60% and 45% ER was achieved for TA and CM at 300$^{o}$C reaction temperature (LT). ER increase to 76% for TA, and to 50% for CM, with an increased in reaction temperature (HT) to at 350$^{o}$C. This increase was found to correspond with biocrude yields and consistent with previous reports as shown in Table 2. For example, Prestigiacomo et al. (2019) reported ER of 44.5% to 57.8% for *Chorella vulgaris*, and 44.7% to 57.2% for sewage sludge when operating at 325$^{o}$C and 30min reaction time.

Importantly, co-liquefaction of CM with TA generally led to improved ER. It was found that more mass of CM in the mixed feedstock led to decrease in ER, the maximum ER was obtained at 04:01 ratio, the minimum at 01: 04. In addition, ER from co-liquefaction of TA and CM at all mass ratio (except for 02:03 and 01:04), and at HT and LT, were found higher than ER mean value of individual liquefaction of TA and CM. Had it been there was no synergistic effect, the ER obtained from co-liquefaction of TA and CM at mass ratio 04:01 (72%), 03:02 (68%) and 01:01(65%) could have been equal to the mean value (62%) of ER from individual liquefaction of TA and TM. At optimal mix ratio of 1:1 for *Spirulina* sp and *Entermorpha pro*. Jin et al. (2013) reported mean value of 45.7% ER, however, 54% ER was achieved for mixed feedstocks.

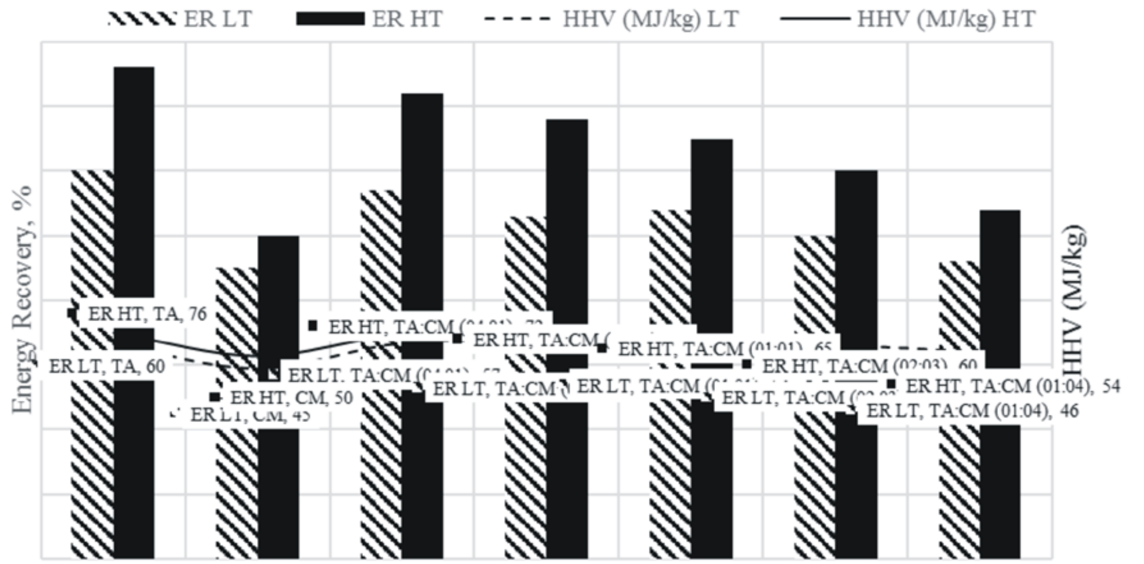


**Figure 5: Energy recovery and higher heating value**

Moreover, biocrudes HHV were found to be between 29MJ/kg to 35MJ/kg. The HHVs suggest that higher carbon led to improve energy density, while higher oxygen content led to decrease in resultant biocrudes. Co-liquefaction of TA and CM seems improve the HHV of biocrudes (31-34MJ/kg) when compared to HHV of biocrude obtained from individual CM (29MJ/kg).The biocrudes HHV were found to be similar to the average HHV of 33.9MJ/kg reported by (Xu et al. 2019) and within range of 32-34.7MJ/kg reported for individual HTL of

*Spirulina* sp. swine manure and digested sludge (Leng et al. 2018). This findings show that synergistic effect occurs during co-liquefaction, improving the quality of biocrude.

## CONCLUSION

This study investigated hydrothermal co-liquefaction of *Tetraselmis* sp. and cow manure for bio-crude production at different feedstock mix ratio. The study showed that co-liquefaction substantially has impact on yield and quality, importantly reducing the nitrogen content of resultant biocrude. Using cow manure as co-feedstock for HTL could be a viable option towards reducing environment pollution while simultaneously producing biofuel.

## REFERENCES

Alba GL, Torri C, Samori C, van der Spek J, Fabbri D, Kersten SRA, Brilman DWF. (2012). Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept. Energy, 26: 642-657.

Baldé H, Vander Zaag AC, Burtt S, Evans L, Wagner-Riddle C, Desjardins RL, MacDonald, JD. (2016). Measured versus modelled methane emissions from separated liquid dairy manure show large model underestimates. Agric. Ecosyst. Environ. 230: 261- 270.

Biller P, Ross AB. (2011). Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. Bioresource Technol. 102: 215-225.

Brilman DWF, Drabik N, Wądrzyk M. (2017). Hydrothermal co-liquefaction of microalgae, wood, and sugar beet pulp. Biomass Convers. Biorefinery. 445–54.

Cantrell K, Ro K, Mahajan D, Anjom M, Hunt PG. (2017). Role of thermochemical conversion in livestock waste-to-energy treatments: Obstacles and opportunities. Ind. Eng. Chem. Res. 46: 8918-8927.

Channiwala SA, Parikh PP. (2012). A unified correlation for estimating HHV of solid, liquid, and gaseous fuels. Fuel, 81: 1051-1063.

Chen W-T, Zhang Y, Zhang J, Schideman L, Yu G, Zhang P, Minarick M. (2014). Co-liquefaction of swine manure and mixed-culture algal biomass from a wastewater treatment system to produce biocrude oil. Applied Energy, 128: 209-216.

Chen X, Peng X, Ma X, Wang J. (2019). Investigation of Mannich reaction during co-liquefaction of microalgae and sweet potato waste. Bioresource Technol. 284: 286-292.

Dandamudi KPR, Muppaneni T, Sudasinghe ST, Holguin FO, Lammers PJ, Deng S. (2019). Co-liquefaction of mixed culture microalgal strains under sub-critical water conditions. Bioresource Technol. 236: 129-137.

Eboibi BE, Lewis DM, Ashman PJ, Senthil C. (2014). Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic Tetraselmis sp. Microalga. Bioresource Technol. 174: 20-29.

Eboibi BE, Lewis DM, Ashman PJ, Senthil C. (2015). Influence of process conditions on pretreatment of microalgae for protein etraction and production of bicorude during hydrothermal liquefaction of pretreated Tetraselmis sp. RSC Adv. 5: 20193-20207.

Eboibi B, Jena U, Senthil C. (2019). Laboratory conversion of cultivated Oleaginous organisms into biocrude for biofuel applications; In: Venkatesh Balan (ed.): Microbial lipid production: methods and protocols, methods in molecular biology. vol. 1995, Humana New York. Springer Nature, 2019.

Feng H, He Z, Zhang B, Chen H, Wang, Kandasamy S. (2019). Synergetic bio-oil production from hydrothermal co-liquefaction of Spirulina platensis and α-Cellulose. Energy. 174: 1283-1291.

Fon-Sing S, Isdepsky A, Borowitzka MA, Lewis DM. (2014). Pilot-scale continuous recycling of growth medium for the mass culture of a halotolerant Tetraselmis sp. In raceway ponds under increasing salinity: A novel protocol; for commercial microalgal biomass production. Bioresource Technol., 161: 47-54.

Gai C, Li Y, Peng N, Fan A, Liu Z. (2015). Co-liquefaction of microalgae and lignocellulosic biomass in subcritical water. Bioresource Technol., 185: 240-245.

Giaconia A, Caputo G, Ienna A, Mazzei D, Schiavo B, Scialdone O, Galia A. (2017). Biorefinery process for hydrothermal liquefaction of microalgae powered by concentrating solar plant: A conceptual study. Applied Energy, 208: pp: 1139-1149.

Hietala DC, Godwin CM, Cardinale BJ, Savage PE. (2019). The independent and coupled effects of feedstock characteristics and reaction conditions on biocrude production by hydrothermal liquefaction. Applied Energy, 235: 714-728.

Huang HJ, Chang YC, Lai FY, Zhou CF, Pan ZQ, Xiao XF, Wang JX, Zhou CH. (2019). Co-liquefaction of sewage sludge and rice straw/wood sawdust: The effect of process parameters on the yields/properties of bio-oil and biochar product. Energy, 173: 140-150.

Isdepsky A, Borowitzka MA. (2019) In-pond strain selection of euryhaline Tetraselmis sp. For reliable long-term outdoor culture as potential sources of biofuel and other products" J Applied Phycology, https://doi.org/10/1007/s1081-019-01873-y

Jin B, Duan P, Xu Y, Wang F, Fan Y. (2013). Co-liquefaction of micro-and macroalgae subcritical water. Bioresource Technol. 149: 103-110.

Lam MK, Khoo CG, Lee KT. (2019). Scale-up and commercialization of algal cultivation and biofuels production. Book Chapter, Biomass, Biofuels and Biochemicals. http://doi.org/10/1016/B978-0-444-64192-2.00019-6.

Leng L, Li J, Yuan X, Li JJ, Han P, Hong YC, Wei F, Zhou WG. (2018). Beneficial synergistic effect on bio-oil production from co-liquefaction of sewage sludge and lignocellulosic biomass. Bioresource. Technol. 251: 49–56.

Prestigiacomo C, Costa P, Pinto F, Schiavo B, Siragusa A, Scialdone O, Galia A (2019) Sewage sludge as cheap alternative to microalgae as feedstock of catalytic hydrothermal liquefaction process. The Journal of Supercritical Fluids, 143: 251-258.

Saba A, Lopez B, Lyam JG, Reza MT. (2018). Hydrothermal liquefaction of loblolly pine: Effects of various wastes on produced biocrude. ACS Omega, 3051-3059.

Sharpley AN (1981). The Contribution of Phosphorus Leached from Crop Canopy to Losses in Surface Runoff. J. Environ. Qual. 10:160- 165.

Tang X, Zhang C, Yang X. (2019). Optimizing process of hydrothermal liquefaction of microalgae via flash heating and isolating aqueous extract from biocrude. J Cleaner Production, https://doi.org/10.1016/j.clepro.2020.120660.

Theegala CS, Midgett JS. (2012). Hydrothermal liquefaction of separated diary manure for production of bio-oil with simultaneous waste treatment. Bioresource Technol. 107: 456-463.

Usapein P, Chavalparit O. (2017). Life cycle assessment of bio-sludge for disposal with different alternative waste management scenarios: a case study of an olefin factory in Thailand. J. Mater. Cycles Waste Manage. 19: 545- 559.

USDA. (2007). Census of agriculture; USDA, National Agricultural Statistics Service, 2009; Vol. 1 ( AC-07-A-51).

Wu X, Liang J, Wu Y, Hu H, Huang S, Wu K. (2017). Co-liquefaction of microalgae and polypropylene in sub-/super-critical water. RSC Adv. 7: 13768-13776.

Wang J, Peng X, Chen X, Ma X. (2019). Co-liquefaction of low-lipid microalgae and starch-rich biomass waste: the interaction effect on product distribution and composition. J Analytical and Applied Pyrolysis. 139:250-257.

Xu D, Wang Y, Lin G, Guo S, Wang S, Wu Z. (2019). Co-hydrothermal liquefaction of microalgae and sewage sludge in subcritical water: Ash effects on bio-oil production. Renewable Energy. 138:1143-1151.

Yang L (Sophia), He Q, Havard P, Corscadden K (Charles), Xu C, Wang X. (2017). Coliquefaction of spent coffee grounds and lignocellulosic feedstocks. Bioresour Technol. 237: 108–21.

Yang J, He Q, Yang L. (2019). A review on hydrothermal co-liquefaction of biomass. Applied Energy, 250: 926-945.

Yuan C, Wang S, Cao B, Hu Y, Abomohra AE, Wang Q, Qian L, Liu L, Liu X, He Z, Sun C, Feng Y, Zhang B. (2019). Optimization of hydrothermal co-liquefaction of seaweeds with lignocellulosic biomass: Merging 2nd and 3rd generation feedstocks for enhanced bio-oil production. Energy. 173:413-422.

Zhang C, Tang X, Sheng L, Yang X. (2016). Enhancing the performance of Co-hydrothermal liquefaction for mixed algae strains by the Maillard reaction. Green Chem. 18: 2542-2553.