

Article

Bio-Crude Production from Protein-Extracted Grass Residue through Hydrothermal Liquefaction

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Abstract: In the present study, the protein-extracted grass residue (press cake) was processed through hydrothermal liquefaction under sub and supercritical temperatures (300, 350 and 400 °C) with and without using a potassium carbonate catalyst. The results revealed that bio-crude yield was influenced by both temperature and the catalyst. The catalyst was found to be effective at 350 °C (350 Cat) for enhancing the bio-crude yield, whereas supercritical state in both catalytic and non-catalytic conditions improved the quality of bio-crude with reasonable HHVs (33 to 36 MJ/kg). The thermal behaviour of bio-crude was analysed and higher volatile contents (more than 50% under the range of 350 °C) were found at supercritical conditions. The overall TOC values in the residual aqueous phase varied from 22 to 38 g/L. Higher carbon loss was noticed in the aqueous phase in supercritical conditions. Furthermore, GCMS analysis showed ketones, acids and ester, aromatics and hydrocarbon with negligible nitrogen-containing compounds in bio-crude. In conclusion, the catalytic conversion of grass residue under subcritical conditions (350 Cat) is favourable in terms of high bio-crude yield, however, supercritical conditions promote the deoxygenation of oxygen-containing compounds in biomass and thus improve HHVs of bio-crude.

Keywords: sub-supercritical temperatures; HTL; grass residue; Bio-crude; aqueous phase



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1. Introduction

The utilization of non-renewable resources, especially fossil fuels, for energy production contributes to carbon emissions, resulting in climate change and global warming concerns. In response, the trend is switching towards renewable energy, which is gaining more attention within the concept of green energy production [1]. Consequently, EU commission is continuously working on the goals of the EU Green Deal to make Europe the first climate-neutral region in the world. A sustainable transportation system using green fuel is one of targets of the EU green deal of 2050. To decrease the concentration of greenhouse gas (GHG) emissions in the transportation sector, the utilization of advanced biofuels is considered as an attractive option. In this case, the production of biofuels from lignocellulosic residues is well known and appealing due to the carbon-neutral cycle, along with waste management [2]. Lignocellulosic feedstocks are ideal resources from different sectors, i.e., agriculture, forest, and other crop fields etc. and are widely used for a multitude of purposes, including the production of biofuels [3]. Different species of grass, such as switchgrass etc., have potential features as dedicated lignocellulosic feedstock that can play a vital role in the bioenergy sector [4–6]. Traditionally, different species of grass residues are used for different applications related to foraging.

There are two main ways to transform grass residue into biofuel products known as thermochemical and biochemical processes by following the different process condi-

tions. It is widely accepted that thermochemical processes show rapid results in terms of biomass decomposition within a shorter time duration than biochemical processes [7]. Hydrothermal liquefaction (HTL) is a well-known thermochemical process that uses high temperature and high pressure for the conversion of different waste residues into renewable bio-crude [8]. HTL works under a subcritical and supercritical water environment in a temperature range of 240–400 °C and pressure of 5–40 MPa [8]. The biomass components undergo certain reactions in the presence of water as solvent such as hydrolysis, decomposition, degradation, and depolymerization, etc., then the molecules are repolymerized into bio-crude fragments, biochar and aqueous phase [9]. The organics present in residual water are termed as water-soluble organics (WSO). The WSO is also considered as an additional product of the HTL process that can be used to reduce the consumption of fresh water on a large scale, as well as to enhance process efficiency in terms of bio-crude yield, especially in the case of lignocellulosic feedstocks.

To date, several studies have been conducted on the thermochemical conversion of different kinds of grasses to recover their potential energy. Anastasakis et al. performed HTL of miscanthus grass with 16 wt.% dry matter and obtained 26 wt.% average bio-crude yield with a great number of different phenolic compounds [10]. Another study was conducted by Patil et al., who processed the residue from the straw crop field and observed 30 wt.% bio-crude yield in the water-alcohol medium [11].

A study conducted by Kumar et al., for the production of bio-crude by using switchgrass in semicontinuous reactors in a temperature range of 235–260 °C noticed 50 wt% of the organic carbon present in the grass was converted to bio-crude after 20 min retention time [12]. Additionally, Cheng et al. also converted switchgrass by using batch reactors under the conditions of 250–350 °C and reported 37% water-soluble product yield under the condition of 250 °C for 1 s [13]. They also identified many lignin-derived compounds, such as phenolic compounds, which appeared in the methanol-soluble fractions, indicating that lignin macromolecules were dissociated, and the resulting compounds could be effectively recovered as methanol soluble products. Furthermore, Zhang et al. also performed HTL of high-diversity grassland perennials and concluded that HTL product yields were dependent upon the process temperature, retention time, and the heating rate of the process. They achieved the highest liquid yield of 82.1% at 374 °C [14].

Jayakishan et al. performed co-liquefaction of *Prosopis Juliflora* and rich paint waste with ratios (1:0, 0:1, 1:1, 2:1 and 1:2) at sub-supercritical range (340 to 440 °C), which resulted in 49% bio-crude yield with energy recovery of 70% and heating values of 46 MJ/kg [15]. Recently, Arun et al. treated the same biomass (*Prosopis juliflora*) in a different medium (NaOH, KOH, HCl and H₂SO₄) in the presence of catalyst (Nano-ZnO) to produce ferulic acid. The optimum yield of vanillin (0.3 g) was found at 70 °C with 0.4% catalyst loading. The optimum bio-crude yield (22.5%) was noticed at a temperature of 300 °C with a biomass-to-solvent ratio of 10 g/200 mL [16].

While a very limited number of publications in the literature deal with the HTL of grass to bio-crude, there has not been a single study in the HTL concerning protein-extracted grass residue. Therefore, the main objective of this study is to assess the potential of protein-extracted grass residue to convert into bio-crude via the HTL process and to explore the process parameters to optimize process conditions towards improved product quality, process performance and product yield. This investigation may play a vital role in terms of waste management and circular economy to establish a sustainable bond between stakeholders and the bioenergy sector.

2. Materials and Methods

2.1. Feedstock Characterizations

The protein extracted grass residue was collected from BioRefine Denmark A/S. BioRefine Denmark A/S is a pioneer in the production of green protein. At their biorefinery, green organic protein is extracted from 3000 hectares of locally produced grass and clover. The plant is expected to produce around 7000 tonnes of organic protein annually with a

crude protein content of over 50%. Green protein extracted from organic grass has great potential as a climate-efficient ingredient in feed for livestock and the long run also food. In Figure 1, the different stages of the process are sketched [17].

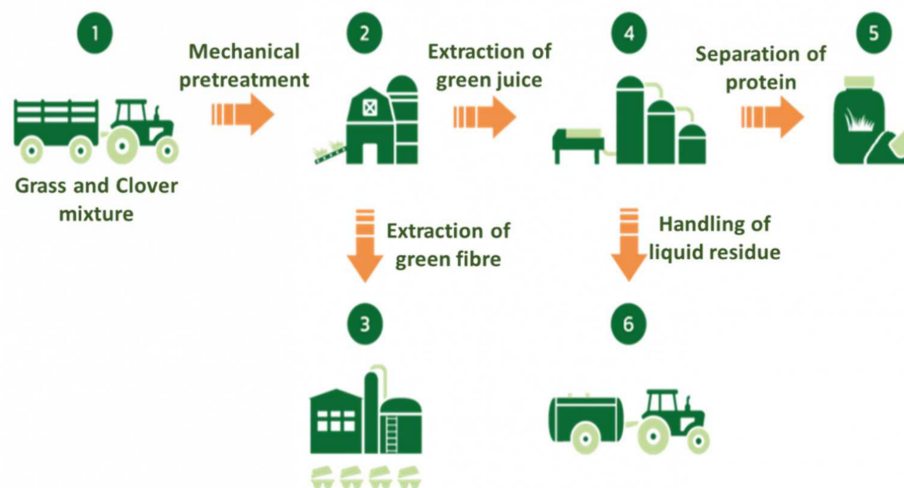


Figure 1. BioRefinery process stages.

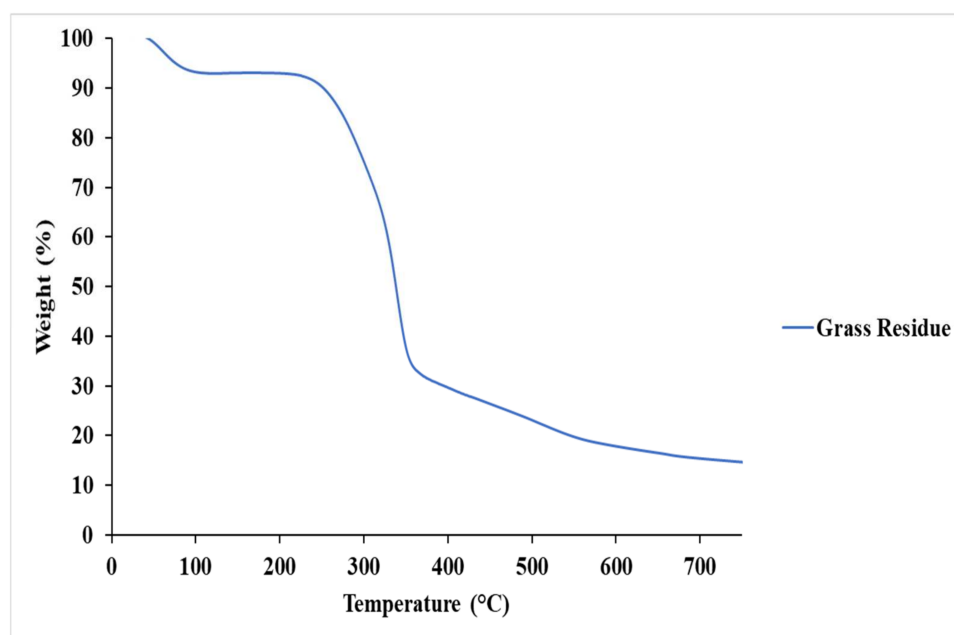
At Stage-1, the raw material primarily grass and clover are cut and mixed in equal proportions (1:1 mass ratio). The raw materials are picked up mostly within 30 km of the plant. At the receiving plant (Stage-2), the raw material is pressed and divided into green juice and green fibre as two separate parts. The green fibre can be used for biogas production (Stage-3) and in the long term it is expected to be used for packaging, textiles, insulation, and other forms of upcycling. The green juice is heated inside the process plant (Stage-4). When the juice is heated, the proteins clump together. After that the green juice is centrifuged, separating the liquid fraction and the proteins in a form similar to coffee grounds. They are fed into the drying room, where the green protein is dried and ends up in big bags (Stage-5). The product can now be used as a diet for chickens, pigs, and cows. The separated brown liquid fraction (Stage-6) from the process plant is spread on nearby fields or used in biogas plants.

For the HTL experiments, the grass residue was collected from Stage-3 of the BioRefine plant and mechanically treated to make the proper slurry for the liquefaction experiments. After that, the initial biomass processing parameters (Table 1) were determined based on the detailed composition of biomass. The moisture content in the grass residue was analysed by the moisture analyser (Kern, MLS) at 120 °C. The ash content was determined through the ASTM D1102 method, (Protherm Furnaces, Turkey) by heating the sample at 775 °C for 4 h. The Higher Heating Value (HHV) of the grass residue was measured through the bomb calorimeter C2000 basic (IKA, Germany), which was found to be close to the theoretical value (HHV = 19.22 MJ/kg), via using Sheng et al. correlation on dried ash-free basis (daf.) [18]. The volatile matter was measured through thermogravimetric analysis (TGA) at 750 °C, under the nitrogen atmosphere. The Elemental analyser (Perkin Elmer, USA, 2400 Series II CHNS/O) was used to analyse the elemental composition. The grass residue contained approximately 42% carbon, with 85% of volatile matter (Figure 2), which indicates that proportion of organic matter to be converted into the bio-crude and other products. The lower amount of nitrogen in the grass residue was due to the extraction of protein. It is a known fact that the lower concentration of ash (3%) and a high percentage of carbon in the biomass are also favourable from an energy recovery perspective.

Table 1. Characterization of grass residue used in the HTL experiments.

Proximate Analysis	
Moisture (%) ^a	8.40
Ash (%) ^b	3.20
Volatile matter (%) ^b	85.34
Fixed carbon (%) ^b	11.46
Elemental analysis (wt.%) ^c	
C	41.69
H	6.49
N	1.43
O ^d	50.40
H/C	1.87
Experimental HHV (MJ/kg)	21.05
Theoretical HHV (MJ/kg)	19.22
Composition analysis (%)	
Cellulose	32
Hemicellulose	27
Lignin	<18

^a As received. ^b Dry basis, ^c Dry ash free, ^d Oxygen calculated by difference.

**Figure 2.** Volatility of grass residue.

2.2. Hydrothermal Liquefaction Experiments

For the HTL experiments, feedstock (grass residue) slurry was loaded corresponding to 20% dry matter with distilled water for a total slurry of 7 g in a 12 mL stainless-steel reactor. All the experiments were carried out in duplicate with and without a catalyst to report the yield with substantial accuracy. Potassium carbonate used in the catalytic run was 2% of the total slurry mixture. After loading, the reactors were closely tightened and purged with nitrogen at 10 MPa pressure to ensure the proper settlement of the slurry. It was observed that without pre-pressurizing, the slurry mixture could not properly stabilize in the reactor and tend to stay in the upper capillary section, which may cause inefficient conversion of biomass [19]. The reactors were then submerged into the pre-heated sand bath at desired temperatures for a reaction time of 15 min. For this study, the designed process temperatures are 300, 350 and 400 °C. The HTL experiments were carried out at a

rapid heating rate, using the preheated sand bath, as rapid heating favours the reduction of char formation. During the experiments, the thermocouples and transducers were used along with the reactors to monitor the process parameters by using the LabView software. The methodology of the experimental setup was adopted by Conti et al. and Shah et al. [8,20].

2.3. Product Separation and Characterization

After the completion of the HTL experiments, the reactors were cooled in the water bucket to room temperature. Firstly, the gaseous product was vented out. Then, the residual aqueous phase containing water-soluble organics was collected without introducing any solvent for the further investigation. The systematic diagram of the HTL process can be referred from Shah et al. [20]. After that, the mixture containing bio-crude and solids were then collected carefully by rinsing the reactors with acetone (Cab Dan, acetone >95%). For the extraction of solids, the liquid was filtered using pre-weighed filter paper (VWR, particle retention: 5–13 μm) by the vacuum filtration technique and dried in the oven at 105 °C for overnight, then weighted to obtain the solid yield. The acetone containing organic phase was evaporated through the rotary evaporator at 60 °C, 56 kPa and a water-immiscible reagent i.e., Diethyl Ether (DEE, Sigma Aldrich, ACS reagent, anhydrous, >99.0%) was added to recover the bio-crude. DEE helps to make a clear layer of bio-crude so that the remaining residual water could easily be removed from the mixture after being put into the rotary evaporator. After the evaporation of DEE, the remaining product is known as HTL bio-crude.

After the separation process, the products were characterized by using different analytical techniques. The elemental analysis of the bio-crude and solids samples were performed by the elemental analyser (Perkin Elmer, USA, 2400 Series II CHNS/O), by operating on CHN mode. However, the heating values of the bio-crude was calculated by using Channiwala and Parikh's correlation [21]. The organic compounds in bio-crude were analyzed through a Gas Chromatograph system coupled with a Mass Spectrometer (GC-MS). The GC-MS spectrometer (Thermo Scientific, USA, Trace 1300, and ISQ-ID, respectively) equipped with a CP-9036 capillary column (5% phenyl 95% dimethylpolysiloxane, 20 m \times 0.15 mm \times 0.15 mm) was employed to characterize the volatile fraction (below 300 °C) of biocrude. For GCMS, the bio-crude samples were prepared by using the DEE and then filtered by using the syringe filters before being injected into GC oven. The pH of the obtained aqueous phase was measured through the pH meter. However, the concentration of the total organic carbon (TOC) and total nitrogen (TN) in the aqueous phase were determined by using the HACH kits. The product yields, energy recovery of bio-crude, and carbon recovery in products were calculated by using the following equations.

$$\text{Bio - crude yield (wt.\%)} = \frac{\text{weight of bio - crude (g)}}{\text{weight of grass residue (g) (daf)}} \times 100 \quad (1)$$

$$\text{Solid residue yield (wt.\%)} = \frac{\text{weight of Solid residue (g)}}{\text{weight of grass residue (g) (daf)}} \times 100 \quad (2)$$

$$\text{HHV(MJ/kg)} = (0.3419) \text{ C (wt.\%)} + (1.1178) \text{ H (wt.\%)} - (0.1005) \text{ O (wt.\%)} - (0.1034) \text{ N (wt.\%)} \quad (3)$$

$$\text{Carbon recovery (\%)} = \frac{\text{wt : \% C in Product}}{\text{wt : \% C in grass residue}} \times \text{product yield (wt.\%)} \quad (4)$$

$$\text{Energy recovery in bio - crude (\%)} = \frac{\text{HHV of bio-crude (MJ/kg)}}{\text{HHV of grass residue (MJ/kg)}} \times \text{bio - crude yield} \quad (5)$$

3. Results and Discussion

3.1. Effect of Temperature and Catalyst on Product Yield

Six experiments were conducted at different temperatures (300, 350 and 400 °C) with and without a catalyst (K_2CO_3), as illustrated in Figure 3. The temperature range (300–400 °C) was selected to investigate the liquefaction behaviour of grass residue under two different mediums

(sub and supercritical) of the water phase. The potassium carbonate (K_2CO_3) was selected as the catalyst due to its higher reactivity than hydroxides in the order of $K_2CO_3 > KOH > NaOH$ [22]. The temperature in the sub-supercritical range decreases the dielectric constant and density of the water, which offers a unique character to the water that can dissolve hydrophobic organic matter [23,24]. Furthermore, potassium carbonate in sub and supercritical water range promotes steam reforming, and water gas shift reactions. Under all temperatures with and without catalyst, the grass residue was successfully transformed into the bio-crude within the yield of 26 to 34% (daf.). It was noticed that in all noncatalytic samples, the temperature did not influence the bio-crude yield as almost 27 to 29% yield was obtained.

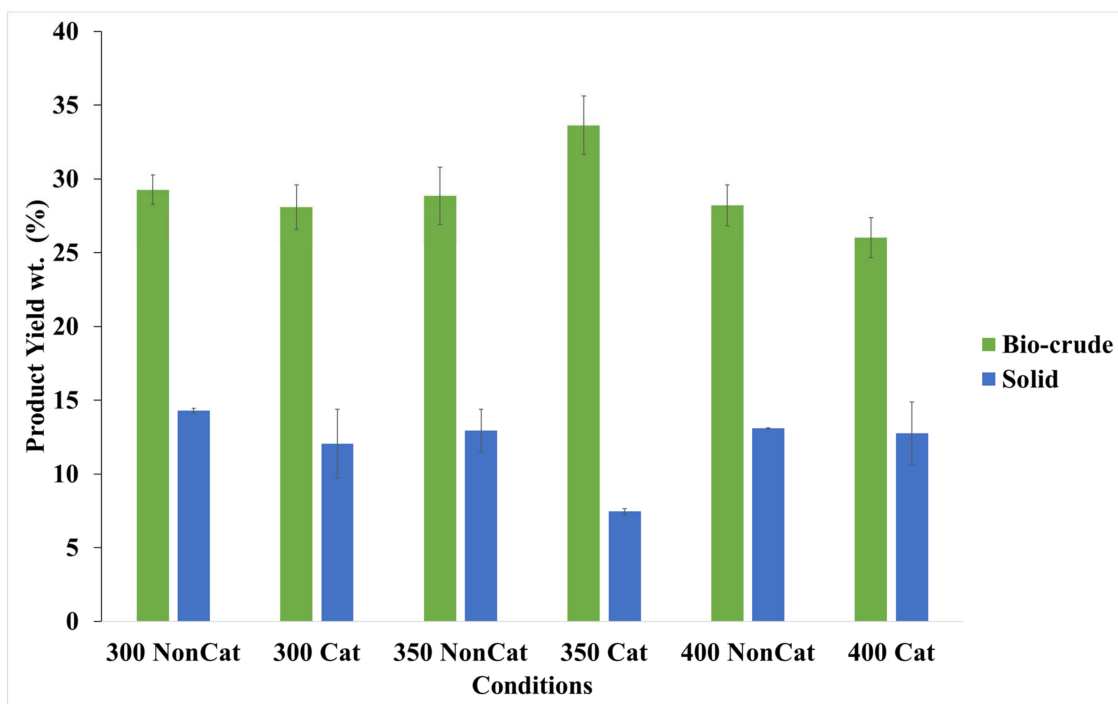


Figure 3. Product yield at Sub-Supercritical conditions.

However, the addition of a catalyst changed the trend in the bio-crude yield significantly. In the subcritical range, the catalyst either increased or stabilized the bio-crude yield. However, the further increase in temperature above the critical point $373\text{ }^\circ\text{C}$ decreased the bio-crude yield. It is a known fact that supercritical temperature above $380\text{ }^\circ\text{C}$ dominates the repolymerization and cracking reactions, which converts organic fractions of biomass into char and gases [25,26]. The high carbon recovery in both solid and gas phases at catalytic supercritical condition ($400\text{ }^\circ\text{C}$) confirms this implication, it is further discussed in Section 3.6.

The catalyst showed a very interesting trend of bio-crude yield with temperature, as the slightly lower bio-crude yield was observed at 300 Cat and 400 Cat. The lower bio-crude production under these conditions might be due to the formation of higher amount of solids and water-soluble organics. The maximum yield was noticed at 350 Cat, which is reported to be the best temperature as per previous studies also [20,26,27].

In the subcritical range from 320 to $350\text{ }^\circ\text{C}$, the alkali catalyst acts as a strong agent for accelerating the hydrolysis reactions via cleavage of glycosidic bonds of the carbohydrates that transfer the majority of organics to bio-crude by suppressing the char formation [28]. The carbonates react with water to form bicarbonates and hydroxides, which are intermediates and serve as secondary promoters for the liquefaction process [29], as given in the following Equations (6) and (7).





The overall solid yield was in the range of 7 to 14%. The formation of solids or char was inevitable due to the occurrence of repolymerization and of lignin and carbohydrates components at lower temperatures. However, the same trend was repeated for 400 NonCat, containing the highest solid yield (14%), which might be due to the higher rate of repolymerization in the supercritical region and the absence of a catalyst. Nonetheless, a lower solid yield was noticed in all catalytic samples, which proves the usefulness of alkali salts as catalysts for biomass conversion. It is reported in several studies that the formation of char is caused by dehydration and polymerization of 5-hydroxymethyl furfural (5-HMF). However, the addition of an alkali catalyst suppresses the dehydration of unsaturated compounds and results in a lower amount of solids [30,31].

3.2. Quality of Bio-Crude

The elemental composition of the bio-crude is given in Table 2. The substantial amount of carbon content in the bio-crude (68 to 77%) was detected through elemental analyser. Higher carbon was detected in the supercritical state at both catalytic and non-catalytic bio-crude with the lowest values for oxygen content. This suggests that the supercritical state promotes a higher rate of deoxygenation through dehydration and decarboxylation reactions. The hydrogen content was consistent at 6 to 8%. No trend in nitrogen was noticed, however, a reasonable concentration (1.19 to 2.40%) in bio-crude is owing to the nature of the lignocellulosic feedstock. The HHV of all bio-crudes were calculated in the range of 31 to 35 MJ/kg. The energy recovery (ER) was also calculated and found to be in the range of 43 to 51%. The maximum ER was estimated in sample 350 Cat due to its higher bio-crude yield. Conclusively, the HHVs of bio-crude were fairly lower than the conventional fossil crude (42 to 44 MJ/kg [32]). Therefore, the bio-crude needs to be upgraded through hydrotreatment to compete with the standards of drop-in fuel.

Table 2. Elemental composition of bio-crude.

Samples	Elemental Composition (wt.%) ^a				H/C	O/C	HHV (MJ/kg)	ER (%)
	C	H	N	O ^b				
Grass residue	41.69	6.49	1.43	50.40			21.36	
300 NonCat.	68.30	7.12	1.23	23.36	1.25	0.26	31.48	43.14
300 Cat.	67.98	7.21	1.19	23.62	1.27	0.26	31.48	41.39
350 NonCat.	71.94	7.48	2.11	18.48	1.25	0.19	33.18	44.82
350 Cat.	71.22	7.47	1.68	19.63	1.26	0.21	32.92	51.85
400 NonCat.	73.02	7.49	2.29	17.21	1.23	0.18	33.58	44.35
400 Cat.	77.48	8.05	2.40	12.07	1.25	0.12	35.82	43.65

^a Dry ash free. ^b Oxygen calculated by difference.

3.3. Volatility of Bio-Crude

The thermal stability of the bio-crude is very much important from a combustion point of view. The thermal behaviour of the bio-crude was analysed through TGA in a nitrogen environment, as shown in Figure 4. Among all the samples, the bio-crudes obtained under catalytic conditions showed a regular trend of higher volatile compounds with an increase in temperature. The observed trend is most probably due to the increased degree of deoxygenation through decarboxylation (-CO₂) and decarbonylation (-CO) pathways with increasing reaction temperature [25]. However, under catalytic supercritical conditions (400 Cat), bio-crude showed the highest weight loss (>70%) of the volatile fragments after 150 °C, which indicated the presence of saturated hydrocarbons with low boiling ranges. The major weight loss of all bio-crudes occurred in a single step. More than half the mass of bio-crude consisted of low (<180 °C) and medium-range (180–350 °C) boiling point compounds, which represent the gasoline and diesel fractions. Similarly, the previously reported TGA results of bio-crude obtained from proteinous grass, i.e., Napier

grass, showed 70% of volatile fractions having boiling range similar to that of diesel [33]. However, under subcritical conditions (300 Cat and 350 Cat), slightly lower weight loss indicated that the lower volatiles fractions were formed due to the lower deoxygenation of biomass at lower temperatures (300 and 350 °C). At an extreme temperature of 800 °C, 23 to 30% of non-volatile fractions were noticed in all bio-crudes, which represent the asphalts and residue. This heavy residue can further be minimized by improving the bio-crude quality via upgrading.

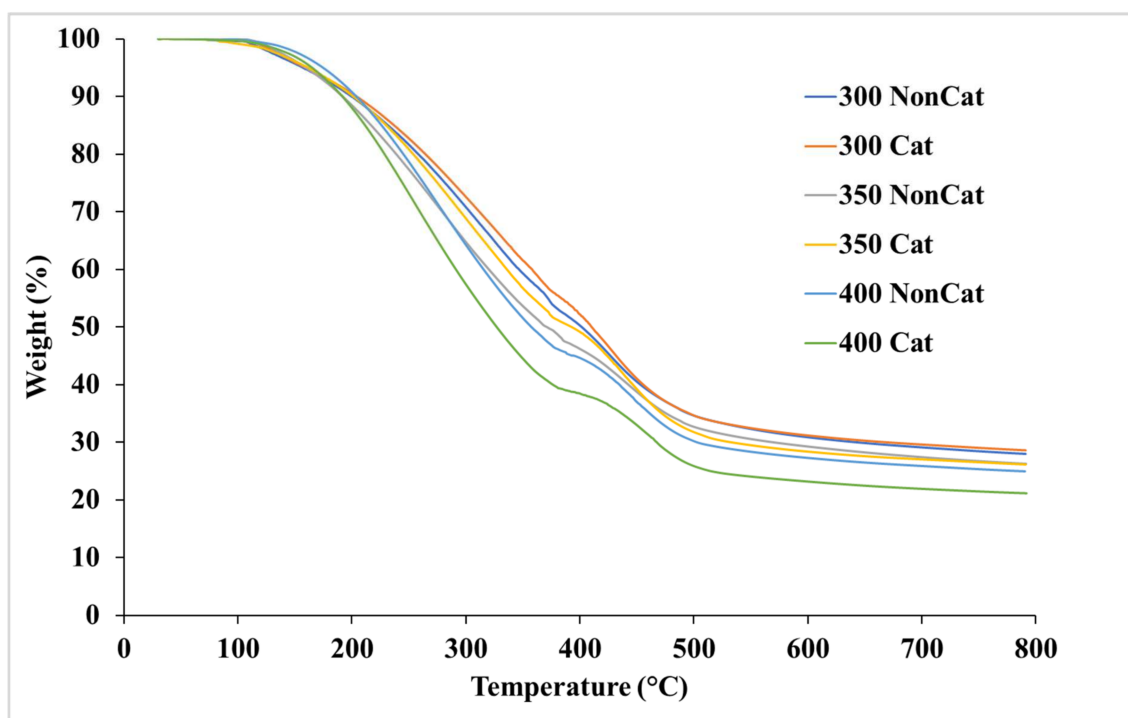


Figure 4. Volatility curves of bio-crudes.

3.4. Composition of Organic Compounds

The compound composition of bio-crude was analysed by GCMS. Although GCMS identifies only approximately 50% of the weight of the bio-crude at the operating temperature of 350 °C, the obtained results are limited to the volatile fractions of the bio-crudes under 350 °C. From 200 compounds, the top 30 compounds were selected, which covered more than 50% area of the total area of compounds. Comprehensive information concerning the detected compounds is provided in Table 3. A variety of compounds were identified and classified into different categories: Alcohols, Acids and Esters, Ketones, Aromatic, Hydrocarbons, and Nitrogen-containing compounds, as illustrated in Figure 5.

At different temperatures with and without a catalyst, the overall chemical composition of the bio-crude was not affected to a greater extent. However, a higher number of aromatics were detected in the supercritical temperature 400 °C at both catalytic and non-catalytic conditions, which might be due to the cleavage of lignin derivatives. In the subcritical range at 350 °C, the lower number of aromatics with higher number of ketones and hydrocarbons indicates the cleavage of hemicellulose and cellulose compounds into their intermediates, which might be converted into ketones, acids, and hydrocarbons by different reactions. The bio-crude obtained at 350 °Cat, also showed similar behaviour, as alkali catalyst readily solubilizes the sugar monomers and convert them into different oxygenates (ketones, alcohols, acids and esters).

Table 3. Compound list of detected compounds.

S. No.	RT	Compounds	Peak Area (%)					
			300 NonCat	300 Cat	350 NonCat	350 Cat	400 NonCat	400 Cat
Ketones								
1	2.71	2-Butanone	1.21	1.41	2.13	2.11	0.97	1.11
2	7.78	2-Pentanone, 4-hydroxy-4-methyl-	1.34	2.14	3.43	2.45	3.14	1.75
3	9.22	2-Cyclopenten-1-one, 2-methyl-	2.12	1.34	1.43	2.56	1.54	0.78
4	10.22	3-Ethylcyclopentanone	1.11	1.56	1.87	2.52	1.01	1.87
5	11.46	4,4-Dimethyl-2- cyclopenten-1-one	2.32	2.12	2.65	2.45	1.34	1.89
6	22.89	2-Nonadecanone	1.98	1.34	1.67	1.54	1.87	0.67
Acids and esters								
7	2.77	Acetic acid	1.43	0.87	1.11	0.75	0.91	1.23
8	16.01	2,5-Octadecadiynoic acid, methyl ester	0.33	0.56	1.78	0.56	0.56	1.23
9	19.51	5-Benzofuranacetic acid, 6-ethenyl	1.73	0.87	1.45	1.26	0.89	0.89
10	29.65	Oleic acid, eicosyl ester	1.12	0.83	1.55	1.32	1.11	0.98
Aromatics								
11	7.51	8 3,5-Dihydroxytolune	3.13	3.98	2.13	2.89	4.55	4.12
12	10.77	Phenol	3.15	3.47	3.15	3.47	3.12	3.47
13	12.05	Phenol, 2-methyl-	3.56	4.76	2.65	2.67	4.65	4.76
14	13.87	Phenol, 3-ethyl-	2.34	2.70	1.98	1.75	2.98	2.70
15	14.29	Creosol	1.15	1.65	1.15	1.65	1.15	1.65
16	15.57	Phenol, 4-ethyl-2-methoxy-	3.23	3.45	1.11	1.45	3.12	3.45
17	16.57	Phenol, 2,6-dimethoxy-	2.45	0.45	2.65	2.68	1.65	3.45
18	8.58	1 1,2,3-Trihydroxybenzene	1.10	1.67	1.11	1.67	2.12	1.67
19	9.27	3 4-[(1E)-3-hydroxyprop-1- en-1-yl]phenol	0.57	0.98	0.68	0.98	0.67	0.98
Hydrocarbons								
20	8.94	2-Octene	1.35	1.67	2.34	2.78	1.32	0.92
21	11.64	1-Methylcyclooctene	1.11	1.23	0.98	2.11	1.42	0.73
22	14.77	1,5,5-Trimethyl-6- methylene-cyclohexene	1.31	1.67	2.34	2.78	1.65	0.96
23	17.12	Hexadecane	1.35	1.67	2.34	2.78	1.32	0.98
24	25.16	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.35	1.67	2.34	2.78	1.32	0.98
N-containing compounds								
25	25.53	Dodecanamide,	0.98	1.12	1.22	0.66	0.86	1.33
26	25.85	N-Methyldodecanamide,	0.89	0.77	0.55	0.67	0.89	0.77
27	27.11	9-Octadecenamamide- (Z)]	0.12	0.56	0.78	0.44	1.32	0.67
Alcohols and Aldehydes								
28	12.81	Cyclohexanol, 3,3,5-trimethyl	1.22	1.45	1.43	2.34	1.45	1.76
29	18.29	1,1-Diphenyl-4- phenylthiobut-3-en-1-ol	1.56	1.78	1.87	2.89	1.98	1.51
30	9.86	cis-4-Decenal	0.98	0.67	0.45	0.67	1.56	1.54
Total area covered			53.23	57.23	59.65	51.21	52.87	57.48

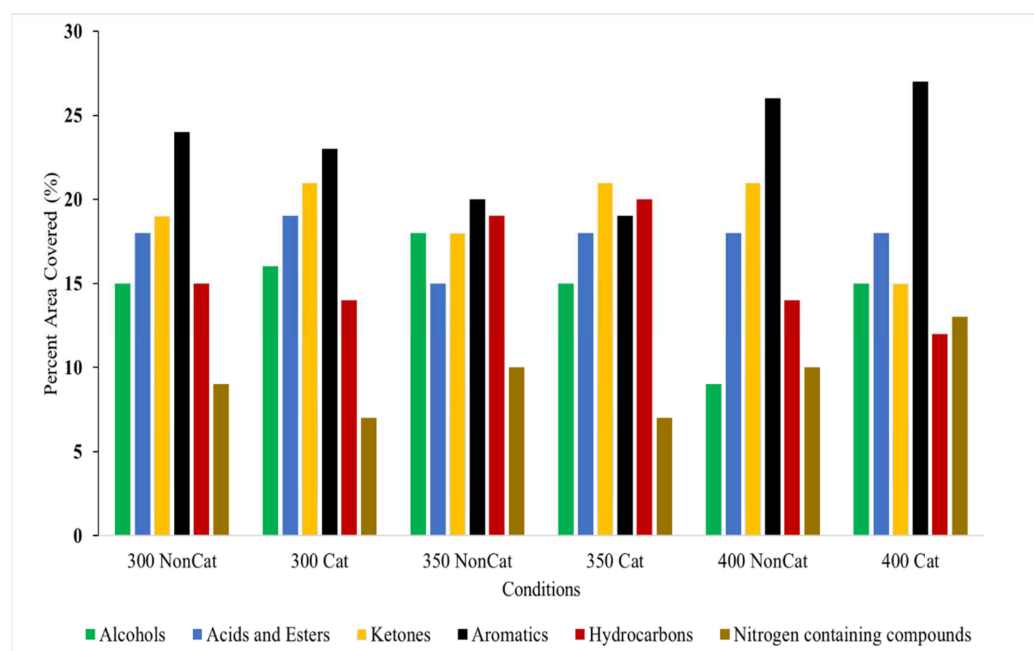


Figure 5. Distribution of Organic Compounds.

It is always difficult to explore the exact reaction pathway of these compounds due to the occurrence of various reactions at the same time. However, we could speculate through their reaction chemistry as the ketones such as (2-butanone, 2-pentanone, 4-hydroxy-4-methyl-, 2-cyclopenten-1-one, 2-methyl-) are produced from the hydrolysis and dehydration of polysaccharides. Aromatic compounds, such as phenols and their derivatives, are primarily originated from the lignin and repolymerization of the long chain compounds from carbohydrates [34]. Acids and esters were placed in a single category such as (acetic acid, 2,5-octadecadiynoic acid, methyl ester, oleic acid, eicosyl ester), might be originated from hydrolysis of carbohydrates and subsequent oxidation of ketones or aldehydes [31]. The nitrogen compounds, which mainly include amides (dodecanamide, N-methyldodecanamide, 9-octadecenamide-Z) were found to be in minor concentrations less than 7%. These amines were derived from the decarboxylation and amination of the monomers of carbohydrates containing nitrogen in the ring structure [35]. Long-chain hydrocarbons such as (2-octene, octadecane, 3-ethyl-5-(2-ethylbutyl)) produced from decarboxylation of carboxylic acids [36]. The summary of GCMS results reveal that bio-crude contained almost 80 to 85% of the oxygenated compounds. Therefore, the removal of oxygen along with nitrogen is very much important to improve the quality bio-crude via adopting cost-effective hydrotreatment techniques.

3.5. Analysis of Solid Residue

Solids also contained a substantial amount of carbon (55 to 63%), with their HHVs of 23 to 27 MJ/kg, as provided in Table 4. The lower H/C values show that little amount of hydrogen was left in solid residue, which might be in the form of aromatic compounds. A high amount of oxygen was inevitable due to the formation and deposition of unsaturated aromatic compounds containing oxygen. Here, the shifting of higher carbon to the solid could lead to an adverse impact on bio-crude productivity. However, higher calorific values of biochar could open up a window of utilizing biochar as a heating source [37], which could be incorporated in the loop of circular economy to establish the sustainability of HTL processing.

Table 4. Analysis of Solid residue.

Samples	Elemental Composition (wt.%) ^a				H/C	O/C	HHV (MJ/kg)	ER (%)
	C	H	N	O ^b				
300 NonCat	63.93	4.91	1.62	29.56	0.92	0.35	27.31	18.28
300 Cat	55.98	4.65	1.53	37.85	1.00	0.51	24.20	13.67
350 NonCat	61.94	4.23	1.75	32.09	0.82	0.39	25.80	15.64
350 Cat	58.30	4.07	1.47	36.15	0.84	0.47	24.34	8.50
400 NonCat	65.24	4.17	1.62	28.98	0.77	0.33	26.89	16.49
400 Cat	57.14	3.59	1.33	37.95	0.75	0.50	23.35	13.96

^a Dry ash free. ^b Oxygen calculated by difference.

3.6. Analysis of Aqueous Phase and Carbon Recovery in HTL Products

The residual aqueous phase was characterized through TOC, TN and pH values. It is known that the formation of bio-crude depends upon the degree of saturation of organic compounds and how efficiently organic matter solubilize into the water phase, which can further go through various reactions such as condensation, repolymerization, cyclization to form bio-crude [36]. The overall TOC in the aqueous phase ranges between 22 to 38 g/L. However, we can see a clear trend of TOC level with reaction temperature, as higher temperature increases the TOC level of the aqueous phase. The maximum carbon loss (higher TOC) in the aqueous phase at supercritical conditions without using a catalyst is due to the supercritical water oxidation of biomass.

On the other hand, the catalyst has not shown any trend with temperature in terms of TOC. We can observe that in the 350 Cat sample, the optimum amount of TOC 35 g/L was recovered to support the maximum formation of bio-crude yield (34%). This suggests that the rise in temperature from 300 to 350 °C, increases the degradation of organic matter and assists the formation of water-soluble compounds, which could repolymerize together to form the bio-crude. However, a further increase in temperature from 350 to 400 °C leads to the gasification of the biomass and results more gaseous products.

Lower temperature at 300 °C, showed TOC 27 g/L, which showed that lower temperature did not allow the hydrophobic matter to intermingle with water and form of WSO. Total nitrogen again varies in small concentrations less than 5 g/L, which indicates the saturation of nitrogen-containing organic compounds (carbohydrates fraction or proteins). pH increases with a simultaneous rise in temperature and the addition of an alkali catalyst. The overall range of pH falls in the acidic range; this is particularly due to the formation of the acids and esters from the decomposition of the grass residue during the liquefaction process. The alkali catalyst increases the pH, and assists in the cleavage of carbohydrates [38].

The carbon recovery in all HTL products was calculated by different methods. For bio-crude and solids, the elemental values were taken corresponding to their yield. For the aqueous phase, the TOC values were multiplied by the amount of water received after the liquefaction experiments. It was observed that from the best possible separation method, almost 70% of water was collected, and the rest was intermingled with bio-crude and solid residue. In order to calculate the accurate carbon recovery for the aqueous phase, it is necessary to have the same amount of water (output = input). The CR in the gas phase was calculated by difference.

Figure 6 shows that 48 to 60% of carbon was recovered in bio-crude. The highest CR of 60% in bio-crude was observed at 350 Cat due to a higher yield, then followed by the solids 10 to 27%, aqueous phase (13 to 20% in the form of WSO), and 10 to 22% in gases that might be in the form of CO₂, CO, CH₄, H₂, etc (Figure 7). However, the gas phase was not analysed in the present study. Several studies reported that the gas phase from lignocellulosic feedstocks contains 60 to 80% of CO₂, the remaining portion was covered with other gases like H₂, CO etc. [18,36].

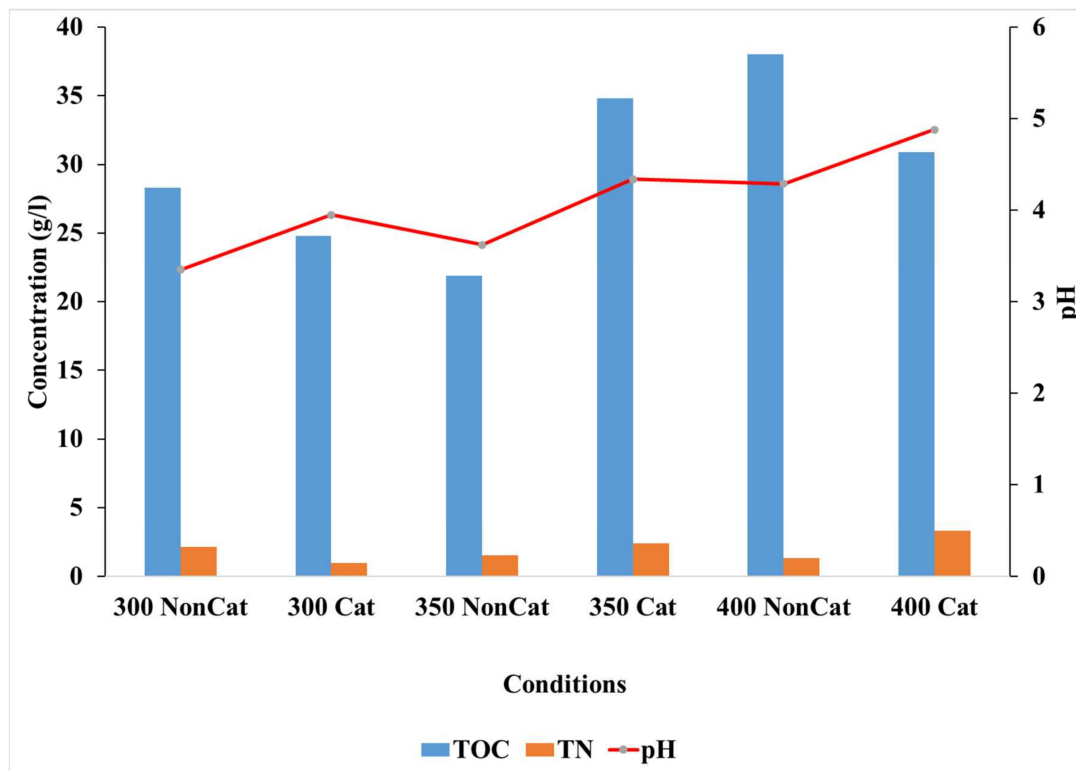


Figure 6. TOC, TN and pH of the aqueous phase.

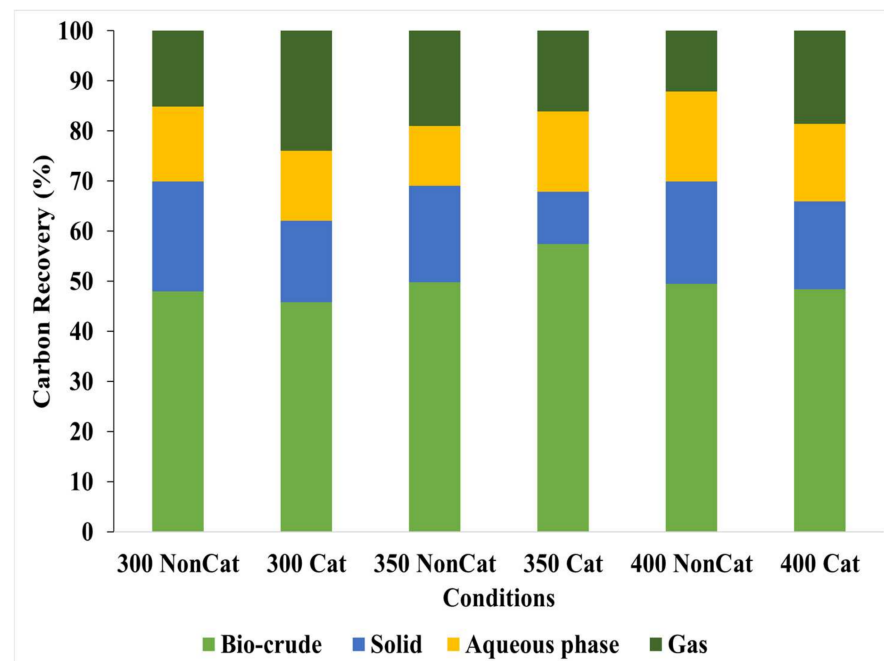


Figure 7. Carbon recovery in HTL products.

Here, 13 to 20% of carbon was lost to the aqueous phase. This loss of carbon decreases the bio-crude productivity and reduces the overall efficiency of HTL processing. Therefore, over the years scientists have tried to valorise the water phase through different routes, such as for anaerobic digestion [39], cultivation of other biomass if it contains nitrogen and phosphorus [40], or for gasification to produce the Syngas [41,42]. All of these approaches require the integration of the HTL unit with other thermochemical or biological technologies.

This step could undermine the overall sustainability of HTL, as it cannot stand alone without integration. In the HTL context, the recirculation of the aqueous phase back to the HTL unit would be the best possible way to recover that energy. Simultaneously, it reduces the freshwater costs for making slurry especially for the dried feedstock such as wood and other lignocellulosic materials. In the recent past, Seehar et al. and Daniele et al. applied recirculation of the aqueous phase with different lignocellulosic biomass (wheat straw and blackcurrant pomace). Both authors noticed higher bio-crude yields with recycling compared to the reference run [26,43]. The application of recirculation could make the HTL process more supportable and economical on a large commercial scale.

4. Conclusions

In this study, the protein extracted grass residue was processed under sub-supercritical temperatures (300, 350 and 400 °C) with and without an alkali catalyst (K_2CO_3). The catalyst was found to be best under subcritical temperatures (350 °C), producing maximum bio-crude yield (34% daf). Whereas the supercritical state improved the quality (HHV) of bio-crude. The collected aqueous phase was in the acidic range (pH: 3 to 5) with higher carbon loss at supercritical states.

In conclusion, catalytic subcritical condition (350 Cat) is favourable for bio-crude production, whereas supercritical condition promotes deoxygenation and improves HHVs of bio-crude. Thoroughly, this study will encourage engineers to design a suitable HTL plant for lignocellulosic biomass by selecting an optimum processing condition. No doubt, a detailed Technoeconomic analysis (TEA) study is still required, which can differentiate the pros and cons of sub-supercritical states to establish the further sustainability of HTL processing.

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