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Co-hydrothermal liquefaction of Phumdi and Paragrass an aquatic biomass: Characterization of bio-oil, aqueous fraction and solid residue

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ABSTRACT

Hydrothermal liquefaction (HTL)of Paragrass (PG) and Phumdi (PH) and a mixture of (PG-PH) a wet aquatic biomass were examined. Reaction parameters such as temperatures (260, 280, and 300 °C) and solvents (water, ethanol, and methanol) were screened on the products distribution and characterization. The maximum bio-oil yield (13.82 wt.%) was observed for PG under water solvent. However, when a mixture of biomass (PG-PH) was used in HTL, the maximum bio-oil yield was 11.66 wt.%under ethanol solvent. While, maximum conversion of 98.4 wt.% was found with water solvent at 300 °C for PH biomass. PH and PG biomass when liquefied alone, majorly produced ester compounds (49.21 and 52.58%under ethanol solvent. Whereas, co-liquefaction bio-oil consisted of phenolic compounds, 67.49% with ethanol as solvent followed by 50.84% in water and 49.59% in methanol.Higher carbon conversion efficiency (69.8%) observed in case of PH biomass under water solvent. The PG-PH liquefaction aqueous fraction showed significantly higher (57000 mg/L) organic carbon. Obvious the difference of products yield and compounds found during liquefaction reaction it's ascribed the distinct effect of solvents and temperatures on biomass macromolecule breakdown.

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1. Introduction

Biomass is the only renewable and sustainable energy source that can replace fossil fuels usedfor the production of fuels/chemicals. Fuel production by thermo chemical conversion from 1st and 2nd generation feedstock has a disadvantage, as these compete with the food for arable area, water and other resources (He et al., 2020; Wang et al., 2018; Raheem et al., 2018). Therefore, there is a need to focus on third-generation biomass feedstocks like aquatic biomass for thermo chemical conversion into value-added fuels/chemicals (Wang et al., 2018; Yan et al, 2019). Aquatic biomass has an advantage over the terrestrial plant biomassdue to their higher growth rate and lesser demand on land surface for cultivation (Yan et al., 2019; Duanetal., 2011; Zhang et al., 2016). One of the major limitations of aquatic biomass is the higher water content, which often makes the process of conversion uneconomical due to the cost and technicalities of the dewatering step. Methodologies that work without the dewatering (drying) step is therefore advantageous and the hydrothermal liquefaction (HTL) process have hence drawn much attention for fuels/chemicals production (Yan et al., 2019;Ong et al., 2018; Wang et al, 2018). In-situ thermal up-gradation of the bio-oil has been accomplished by use of different solvents or by addition of H_2 and/or catalyst to remove oxygen and nitrogen, to improve the heating value (Duan et al., 2011; Leong et al., 2018).With water solvent, the yield of bio-oil is usually lower compared to the alcoholic solvent used in hydrothermal liquefaction (Yang et al., 2019; Biswas et al., 2017).The alcoholic solvents have lower boiling points and critical points than water, which can dissolvemore efficiently the organic products (Han et al., 2019; Biswas et al., 2017).

Co-liquefaction with different solvents is shown to promote thebio-oil yield and selectivity of compounds (Jin et al., 2013; Chen et al., 2015). Nevertheless, there have been no studies on the hydrothermal liquefaction of mixed aquatic biomass feedstock in different solvents. Jin et al. (2013) studied co-liquefaction under the subcritical water solvent, where the biomass from *Spirulinaplatensis* (SP) and the macroalga *Entermorphaprolifera* (EP) was reacted at 340 °C either individually or asSP-EP mixtures. They varied the biomass ratio of 0 to 100% and the maximum bio-oil was observed in a 50:50 mixture of SP-EP was used in

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the HTL reaction. They also studied the quality of the bio-oil and showed that the elemental N_2 content in 50:50 mixture bio-oil was maximum. Bio-oil composition analysis showed that the compounds obtained from EP bio-oil were more comparable to the mixture bio-oil rather than the SP bio-oil (Jin et al., 2013). Chen et al. (2014) investigated the co-liquefaction of the mixtures of microalgae and swine manure at different ratios for 60 min at 300 °C. They observed that the highest bio-oil recovery of 35.7% was obtained at an increased swine manure concentration of 75.0% (Chen et al., 2014). Gai et al. (2015) conducted co-liquefaction of microalgae and rice straw biomass mixtures at different temperatures (200-350 °C) and reaction holding times (10-90 min.). They observed that as the microalgae biomass increases from 0 to 100 %, the bio-oil recovery was higher and peaking at 43.6 wt.%. The bio-oil composition analysis showed that with an increasing amount of microalgal biomass, there is an increase in the nitrogen-containing functional groups (amides and N&O heterocyclic) whereas,the oxygenated functional compounds such as ester, ketones, etc. decreased (Gai et al., 2015). Co-liquefaction of a mixture of algal biomassfrom *Cyanidioschyzonmerolae* (CM) and *Galdieriasulphuraria* (GS) at 300 °C for 30 min under H_2O solvent was examined by Dandamudi et al. (2017). The maximum bio-oil yield was obtained when a CM/GS ration of 80:20 was used. The composition of bio-oil from the biomass mixture did not change in comparison to that obtained from individual algal biomass liquefaction (Dandamudi et al., 2017). The co-HTL of *Spartinaalterniflora* (SA) and *Spirulina*sp (SP) was carried out by Feng et al. (2018) at different temperatures (260-360 °C) for 30 min. under both water and water-ethanol as solvents.The highest bio-oil (45.63 wt.%) was observed at 340°C with a higher heating value of 34/ MJ/kg. The higher hexadecenoic acid ethyl ester obtained was same as that obtained from individual biomass liquefaction (Feng et al., 2018). Xu et al. (2017) carried out the co-HTL of microalgae (*Chlorella*) and sewage sludge (SS) at 340 °C in a hydrogen environment for 30 min. reaction holding time. They observed maximum bio-oil yield of 4.7wt % with 1:1 ratio of biomass, while solid residue yield decreased to 3.6 wt.%. Addition of SS residue in the liquefaction reaction resulted in decreased content of the elemental oxygen and nitrogen, while the carbon and hydrogen content increases. Hence, co-liquefaction of biomass might improve the bio-oil quality and quantity (Xu et al., 2017).

Loktak Lake in the north eastern state of India, Manipur, is a unique ecosystem where there are floating biomass island called phumdis (Singh et al., 2014). The phumdi vegetation is a mixed lot, but with a significant representation of the invasive paragrass (*Brachiariamutica*) which grows on the phumdis as well as on the lake borders, besides several aquatic plants (Saoo et al., 2017). Lake eutrophication has lead to overgrowth of the invasive plants and there are periodic efforts to mechanically harvest these and clear the lake. As the separation of biomass types is difficult in mechanical harvesting and there are transportation and logistics challenges, it is desirable to have in situ processing of the biomass. Hydrothermal liquefaction of a mixed biomass feedstock (co-HTL) can extensively diminish the logistics costs connected with collection and transportation. Therefore the current study has explored the possibility of a co-HTL of the mixed biomass feedstock, PH, PG, and their mixtures (PH-PG) were characterized, and were treated at different temperatures (260-300 °C) and with different solvents (water, ethanol, and methanol). The liquefaction products were analyzed using Fourier Transform-Infrared spectroscopy (FT-IR), Gas-Chromatography/Mass-Spectroscopy (GC/MS), Nuclear Magnetic Resonance Spectroscopy (NMR), Total organic carbon (TOC), and Scanning electronic microscopic (SEM). The obtained aqueous phase was analysed for the presence of carbon and nitrogen elements.

2. Materials and Methods

2.1 Feedstock and products analysis methods

Phumdi(PH) and Para grass (PG) aquatic biomass were collected from the Loktak Lake, Manipur, India. The wet aquatic biomass was used for hydrothermal liquefaction reaction.To evaluate the thermal decomposition characteristics of the biomass samples, thermogravimetry analysis (TGA) and derivative thermogravimetry analysis (DTGA) were performed using a DTG 60 unit (Shimadzu, Japan). Degradation was analysis from ambient temperature to 900 °C in N_2 gas flow with the heating rate of 10 °C/min. Ultimate analysis (elemental analysis) has been carried out with using of Elemental vario microcube unit. Similar to the ASTM D3175 method, proximate analysis of the biomass was characterized by the help of furnace. Proton NMR unit Bruker Avance 500 Plus was used to investigate different protons types present in bio-oils, where the bio-oils are dissolved in $CDCl_3$. The functional compounds present in the bio-oils have been analyzed using GC/MS Agilent 7890 B instruments. DB-35MS column (30 m × 0.32 mm × 0.25 μm) column was used. After injected the bio-oil sample, the column was heated from 50 °C to 280 °C with a heating rate of 5 °C min^{-1} in the presence of Helium (He) as a carrier gas with flow rate of 1ml min^{-1}in a split mood. FT-IR instrument NEXUS670-FT-IR was used for functional group analysis of feed, bio-oil and bio-char. Total organic carbon (TOC) analyzed by SSM-5000A solid sample module. The surface morphology, scanning electron microscope (SEM) of the bio-char characterized by using FEI Quanta 200F instruments.

2.2 Hydrothermal liquefaction reaction and separation procedure

High-pressure autoclave reactor (Parr reactor, 100 ml) was used for the HTL experiments. The HTL reaction was conducted to investigate optimal parameters, which included reaction temperature (260, 280 and 300 °C), solvent (water, ethanol and methanol), single biomass (PG and PH) and mixture of biomass (PG-PH, ratio1:1). The reactor was loaded with biomass and solvent (Ratio1:6 by weight). The air inside was removed by purging nitrogen gas. The reactant in the HTL reactor was stirred at ~300 rpm. After the desire temperature was attained, the temperature was held constant for 15 min.The non-condensable gases werevented out. Solution ofbio-oil and bio-charwas alienated by filtration using extractive solvent diethyl ether for water solvent and alcohol for alcoholic solvent. This fraction of bio-oilwas designated as bio-oil1. The bio-oil extracted from the solid resides (bio-char) by soxhlet extraction with acetone solvents was denoted as bio-oil2. The sum of bio-oil1 and bio-oil2 was chosen as total bio-oil. The unreacted biomass (solid residue) was driedat 80 °C for overnight. Experiments were performed either in duplicates or triplicates and average liquid yields were recorded. Product yields are calculated by the using various equations as given below.

$$Conversion\ (\%) = \frac{F1 - F2}{F1} \times 100$$

$$Bio - oil\ yield\ (wt.\%) = \frac{F\ ether\ soluble}{F1} \times 100$$

$$Solid\ residue\ yield\ (wt.\%) = \frac{F2}{F1} \times 100$$

$$Gas\ yield\ (wt.\%) = (F(vessel + feed + water)\ before\ HTL - F(vessel + feed + water)\ after\ HTL)/(Amount\ of\ feed\ taken\ (g) + amount\ of\ water\ added\ (g)) \times 100$$

$$Other\ yield\ (wt.\%) = 100 - (bio - oil + solid\ residue + gas)$$

F1, is the weight of PH or PGfeed; *F ether soluble*, is the weight of ether soluble bio-oil (bio-oil). *F2*, is the weight of bio-residue.

3 Results and Discussions

3.1 Characterization of feedstock

Raw biomass analysis showed that, PH, PG and mixedPG-PH biomasses hadvery high volatile matter (VM) contents of 73.84%, 80.20% and 78.44%, respectively (Table 1). Higher the VM, higher is the conversion of biomass to products, and thus these biomass feedstocksmay be considered as excellent energy sources for fuels production. PH hada higher amount of ash (11.54 wt.%) and fixed carbon (14.62 wt.%) content compared to the PG. The distinct amount of volatiles, ash and fixed carbon present in the biomass can lead tovariations in the product profile.The TGA/DTGA of three feedstock; PH, PG and PH-PG (ratio1:1) were analyzed and shown in figure 1. The details of the TGA characterization are provided in our previous report (Ayushi et al., 2019). At a temperature range between 200 to 400°C, all three biomass degraded maximum. At higher temperature, the PH-PG 1:1 mixture exhibited maximum decompose DTG peaks compared to either PG and PH alone. It could be due to the compostion and proximate content of the individual feedtsocks showing synergistic effects, which is increased with the degradation temperature. FT-IR analysis of raw biomass was shown in our previous publication (Ayushi et al., 2019).

Table 1. Proximate analysis of PH, PG and PH-PG 1:1

Proximate analysis, wt%	Loktak Biomass		
	PH	PG	PH-PG (1:1)
Moisture	5.90	6.10	5.40
Volatile matter	73.84	80.20	78.44
Fixed carbon	14.62	11.40	12.00
Ash	11.54	8.50	9.60

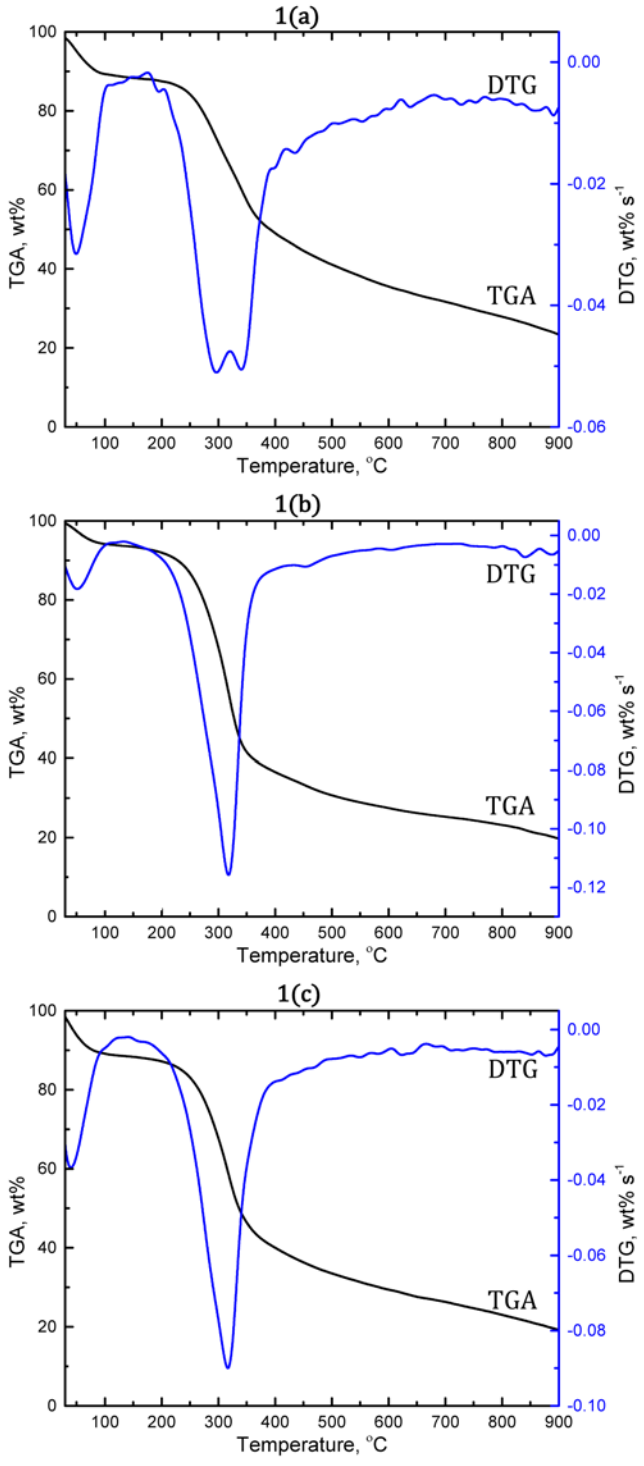

Figure 1: TGA/DTGA analysis of (a) phumdi, (b) para grass, and Phumdi and para grass mixture.

3.2 HTL Products yield

3.2.1 Effect of temperature on product yield

HTL runs for PH and PG were conducted at three temperatures in the range of 260-300 °C under nitrogen atmosphere with a retention time of 15 min. The product distribution is presented in Table 2. Here wet feedstocks are taken for the HTL reaction and moisture present in the biomass ca. 75-78%. It can be observed from the table that the bio-oil yield for both the feedstocks (PG and PH) increased with an increase in temperature. The observed bio-oil yield wt.% for PH was lower compared to PG. The maximum yield of 1.57 wt.% for PH and 13.82 wt. % for PG was obtained at the reaction temperature 300 °C. The range of bio-oil yields was consistent with the previous reported values on aquatic biomass that vary based on nutritional profiles and conversion conditions (Tanget al., 2020; Li et al., 2018). It was showed earlier that the bio-oil yield depends on the biomass compositionand the trend on the bio-oil yield was lipid>protein>carbohydrate. Hence,biomass with higher protein content produced higher bio-oil (Biller and Ross, 2011). The higher bio-oil yield is likely due to hydrolysis and cracking of the macromolecule; enhanced at higher temperature (Han et al., 2019). The yield of gases in both the feedstocks remained low and decreased at a higher temperature.In the case of PH,the yield of gases was observed to be in the range of 2.28-4.71 wt.%, whereas for PG it ranged between 3.09-10.47 wt.%. Bio-char yield decreased with an increase in temperature. The maximum yield of bio-char 6.4 wt.% and 15.33 wt.% was obtained for PH and PG respectively at 260 °C. Role of temperature in the product distribution is influenced by various competing reactions during HTL viz. fragmentation, hydrolysis, and repolymerization. In the present case, it can be observed that the rate of depolymerization increases with temperature resulting in higher bio-oil yield at 300 °C.These results are in agreement with the findings that glucose and protein mixture enhanced the liquefaction temperature for bio-oil yield (Tanget al., 2020; Zhang et al., 2015). The decreased yield of gases and bio-char can be attributed to the occurrence of limited number of secondary reactions at the later stages. The yield of water-soluble oxygenated hydrocarbons (other yield) formed due to decomposition of the holocellulose fraction of biomass, displayed a different trend. For PH, wate-soluble hydrocarbons yield found to be increased up to 94.55 wt.% with the raisein temperature. However, in the case of PG the water-soluble hydrocarbons yield improved up to 81.3 wt.% at 280 °C and then declined. The maximum yield of water-soluble hydrocarbons can be attributed to the occurrence of hydrolysis reactions with the higher rate. The maximum conversion was found for PH and PG biomass as 98.4 and 94.5 wt.% at reaction temperature of 300 °C and 280 °C, respectively.

3.2.2 Effect of solvent on product yield

The material balance obtained from the individual HTL runs using different organic solvents (ethanol and methanol) are presented in Table 3 at an optimum temperature of 300 °C. An advantage of using alcoholic solvents-methanol and ethanol here, for HTL is that they are the renewable chemicals that are derived from biomass and can be recycled and reused after the HTL operation (Yang et al., 2019). The yields were compared with the results obtained with water as a solvent. It is found that, total bio-oil yield for PH increased in the presences of organic solvents. Methanol as a solvent gave the maximum bio-oil yield up to 2.17 wt.% followed by 1.67 wt.% in ethanol and 1.57 wt.% in water. However, for PG, the maximum bio-oil yield of 13.82 wt.% was acquired with water solvent. Use of methanol yielded 7.67 wt.% and 6.34 wt.% of bio-oil was obtained with ethanol as a solvent. The maximum bio-char recovery of 14.9 wt.% was obtained for PG with ethanol whereas, methanol and water yielded 12.59 wt.% and 6.66 wt.% bio-char. For PH, the yields were lower than PG. Methanol gave the maximum yield of bio-char up 5.0 wt.% followed by ethanol and water which gave yields of 3.01 wt.% and 1.6 wt.% respectively. Gas yields were also lower for PH compared to PG. Maximum gas yield of 11.67 wt.% was observed for PG in methanol whereas, ethanol and water gave the gas yield of 6.67 wt.% and 3.09 wt.% respectively. For PH, the maximum gas yield of 10 wt.% was obtained with ethanol followed by methanol and water, which gave maximum yields of 9.28 wt.% and 2.28 wt.%, respectively. Maximum conversion 98.4 wt.% and 93.33 wt.% was obtained for PH and PG respectively with water. While opposite trend was found in case of organic solvent. Similar was the case for water-soluble hydrocarbons (other yield). Maximum other yield of 94.55 wt.% and 76.43 wt.% was obtained for PH and PG with water, probably due to the highernumber of hydrolysis reactions with water compared to solvents. Application of organic solvents other than water results in possible alkylation and esterification reactions

Table 2. Effect of reaction temperature on products yield

Temp, °C	Bio-oil%		Total Bio-oil, wt%	Gas wt%	Biochar, wt%	Conversion, wt %	Other yield, wt%
	Bio-oil 1	Bio-oil 2					
Phumdi							
260	0.2	0.3	0.5	4.71	6.4	93.6	88.39
280	0.4	0.4	0.8	4.00	4.8	95.2	90.4
300	0.17	1.4	1.57	2.28	1.6	98.4	94.55
Para grass							
260	1.67	1.50	3.17	10.47	15.33	84.67	71.03
280	1.83	4.67	6.50	6.67	5.51	94.5	81.33
300	11.66	2.16	13.82	3.09	6.66	93.33	76.43

Table 3. Effects of different solvent on products yield

Solvent	Total Bio-oil, wt%	Gas, wt%	Biochar, wt%	Conversion, wt%	Other yield, wt%
Phumdi					
Water	1.57	2.28	1.6	98.4	94.55
Methanol	2.17	9.28	5.0	95.0	83.55
Ethanol	1.67	10.0	3.01	97.0	85.32
Para grass					
Water	13.82	3.09	6.66	93.33	76.43
Methanol	7.67	11.67	12.59	87.41	68.07
Ethanol	6.34	6.67	14.9	85.0	72.09

between solvents and liquefied biomass intermediates. Moreover, lower dielectric constants of organic solvents, enhances the dissolution and stabilization of the reaction intermediates (Biswas et al., 2017; Han et al., 2019).

3.2.3 Effect of solvent on PH-PG mixture on product yield

The hydrothermal liquefaction of PH and PGmixtures using different solvents (water, ethanol and methanol) were compared. The products distributions are provided in Table 4. From the products yield, it is noticed that maximum bio-oil and bio-char yield of 11.66 wt.% and 7.5 wt.% was obtained in ethanol solvent. Methanol as a solvent gave higher bio-oil and lower bio-char yield than water. The gas yield decreased with the use of alcoholic solvents. The maximum gas yield of 10.71 wt.% was observed with water. The highest conversion of 95.84 wt.% was observed with water. However, the water-soluble hydrocarbons (Other yield) were observed in maximum quantity of around 84.67 wt.% with methanol followed by water (76.30 wt.%). The different behavior of PH-PG liquefaction in subcritical water and supercritical alcohol is proposed to be due to the distinction in hydrogen production activity, reactivity, dielectric constant, and solubility of various products in these solvents (Caporgno et al., 2016; Peng et al., 2016).

3.3 Bio-oil characterization

3.3.1 GC/MS analysis

Gas chromatography-Mass spectrometry (GC-MS) assisted in the categorization of all the compounds in the bio-oil. This gives further insight to all the promising pathways accountable for the breakdown of biomass during HTL and the mixture during co-HTL. Figure 2a (PH) shows the compounds present in the bio-oil, which is obtained from different solvents medium liquefaction at reaction temperature of 300 °C. The graph displays the presence of large percentage of esters in the bio-oil.9-Octadecenoic acid, methyl ester, (E), Methyl 18-methylnonadecanoate was the major ester compound present in the bio-oil. Maximum up to 49.21% with ethanol followed by 47.32% and 31.72% in methanol and water respectively of such compounds were obtained. Aromatic compounds constituted the second major portion in PH bio-oil. 14.95% of such compounds were observed with water whereas; just 1.85% and 0.84% were found in methanol and ethanol respectively. Hydrocarbons such as 1-Cyclohexylnonene showedan area percentage up to 4.71% in water and 0.97% in ethanol. Acids such as 3-Pyrrolidinecarboxylic acid, 1-methyl-5-oxo-, (9-Oxo-9,10-dihydroacridin-4-yl) acetic acid were present with very low area percentage of 1.50% in water. No acids were found in the bio-oil obtained from ethanol and methanol.

Figure 2b (PG) shows the area percentage of the major compounds present in the PG bio-oil obtained with different solvents such as water, ethanol, and methanol at an optimum HTL temperature of 300 °C. The graph displays a greater number of compounds than observed in PH. The maximum portion of bio-oil up to 51.31% was constituted by aromatic compounds in water, followed by 24.02% in methanol, whereas no aromatics were formed with ethanol. Esters were observed to be the second major constituent of bio-oil. Around 52.58% of such compounds were found in ethanol and 14.76% in methanol. Contradictory to PH, none of the ester compounds were obtained in bio-oil prepared with water as a solvent. Compounds containing nitrogen were present witharea percent of 10.19% in ethanol, 3.51% and 2.64% in water and methanol respectively (Li et al., 2018). N-containing compounds are formed due to the decomposition of protein from the Maillard reaction (Tanget al., 2020; Zhang et al., 2015). Hydrocarbons were also formed with water and methanol up to 1.1% and 3.28% respectively. Acids were observed to be present in minute quantity with area percent of 1.4% with ethanol. Bio-oil obtained with water and methanol contained no acidic compounds.

Compounds were found from the HTL of biomass mixture with different solvents at an optimized temperature of 300 °C are displayed in Figure 2c (PG-PH). Phenol such as 2-methoxy-Phenol, 4-ethyl-Phenol, 2,6-dimethoxy-phenol were observed to be the major constituent in all the bio-oils. Maximum area percent up to 67.49% was obtained with ethanol followed by 50.84% in water and 49.59% in methanol. Phenols are formed due to the depolymerization, and reformation reactions of the biomass composition (mainly carbohydrates) during the high pressure conversion (Zhang et al., 2015; Yang et al., 2015). The presence of high percentage of phenol in Co-HTL bio-oilin comparison to the individual yields depicts a synergistic effect. The higher aromatic phenolic compounds were observed during co-hydrothermal liquefaction between

Table 4. Effects of solvent on PH-PG biomass mixture on products yield

Temp, °C	Bio-oil%		Total Bio-oil, wt%	Gas wt%	Biochar, wt%	Conversion, wt %	Other yield, wt%
	Bio-oil 1	Bio-oil 2					
Water	7.17	1.67	8.84	10.71	4.15	95.84	76.30
Methanol	9.67	-	9.67	4.52	1.14	92.0	84.67
Ethanol	11.66	-	11.66	8.09	7.5	92.5	72.75

PH and PG noted that the interaction between PH and PG provoked the aromatization reaction (Hu et al., 2018). Esters were present up to 6.81% in the ethanol and 22.83% in methanol whereas, with water, esters were absent. Aromatics were seen to be present with area percent of 17.77% in water and 5.17% in methanol, and the same were found to be absent with water. Hydrocarbons like 2-Tetradecene, (E), 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-constituted the small portion of bio-oil. 4.43% was seen in ethanol and 2.21% was observed with water. The hydrocarbons identified by GC-MS, were formed from the decomposition of the lipid (Barreiro et al., 2013). Ketones were also present in the bio-oil up to 14.41% in water and 3.2% in methanol. Moreover, in all bio-oil other compounds such as acids, alcohols were constituted with small amount (Lin et al., 2020; He et al., 2020; Toor et al., 2011). The presence of solvent simulates certain solvolysis, hydration, and pyrolysis reactions, which helps in achieving better fragmentation of biomass and enhancing disintegration of reaction intermediates.

3.3.2 ^{1}H NMR analysis

NMR analysis is a powerful tool for the indication of the types of protons present in the bio-oils. Proton percentage of bio-oils from HTL reactions under optimal conditions for PH, PG, and mixture of PG-PH with different solvents (water, ethanol and methanol) are shown in Figure

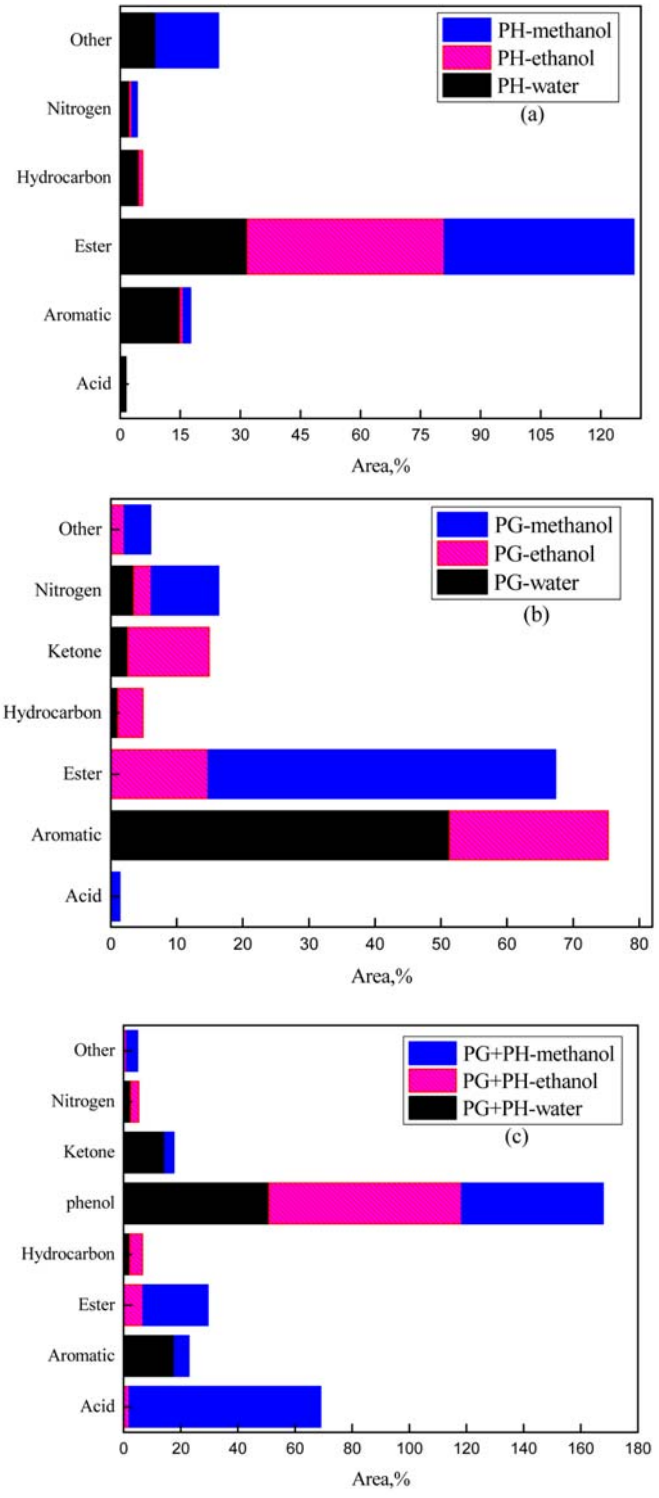

Figure 2. Hydrothermal liquefaction compounds distribution of (a) PH, (b) PG and (c) PG-PH mixture bio-oil.

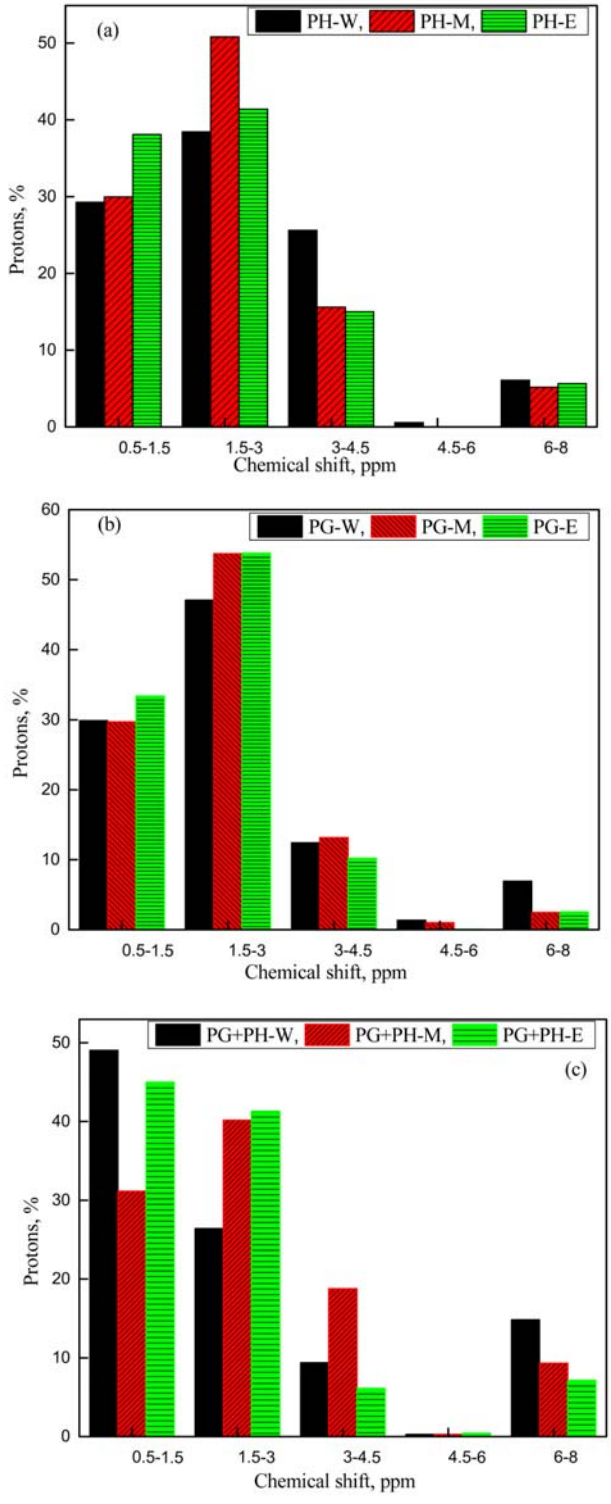

Figure 3. ^{1}H NMR analysis of the hydrothermal liquefaction of (a) PH, (b) PG and (c) PG-PH mixture bio-oil.

3 (a,b and c).Aliphatic protons bonded with heteroatom O or Non are confirmed from region of 0.5 to 1.5 ppm (Mullen et al., 2009). This protons percentage showed in the bio-oil in the range of 29.26-49.04%.It has been observed that the mixture of PG-PH biomass liquefaction bio-oil showed a higher proton percentage (31.15-49.04%). In this region, the bio-oil obtained with PG-PH mixture and water solvent showed a higher amount of protons (49.04%) compared to the other bio-oils. The aliphatic protons attached to the heteroatom and aromatic carbon are conferred in region at 1.5-3.0 (Yan et al., 2019). The bio-oil from individual HTL of PH and PG biomass showeda greateramount of protons in this region (38.45-53.78%) compared to the mixture of PG-PH HTL bio-oil (26.40-41.30%). Alower amount of protons were observed in bio-oil from HTL with water solvent of PH-PG mixture (26.40%), while a higher proton was observed in HTL with ethanol solvent of PG bio-oil. At region from 3.0-4.5 ppm pointed toward the presence of methylene protons or ether protons in bio-oil. An oxygenated compound which is present at higher level (25.62%) in bio-oil from PH HTL with water solvent, whereas the proton amount was decreased in the PG and PG-PH mixture HTL bio-oil.

A large number of functional hydrogen protons and ether protons were ascribed in the range of 4.5 to 6.0 ppm (Yan et al., 2019). These types of molecules were observed at very less quantities in all the bio-oils. The aromatic protons present in the bio-oil were confirmed by peaks observed in the region of 6.0to 8.0 ppm (Byambajav et al., 2018). Results show that lowest was in bio-oil of PG HTL with methanol (2.47%) and highest inbio-oil of PG-PH mixture HTL with water (19.99%). This indicates the presence in bio-oil of aromatic phenolic derivative compounds and this result is in accordance with GC-MS data. The downfield region from 8-10 ppm did not show any spectra in any bio-oil.

3.3.3 FT-IR analysis

FT-IR analyses of the bio-oils obtained at optimal conditions are provided in Figure 4 (a, b and c). The peak observed at 3200-3400 cm^{-1}ascribed the N-H and O-H stretching functional group present of the amides/amines, alcohols and phenols compound (Linet al., 2020; Wang et al., 2019). The alkyl (alkanes) C-H stretching vibrations bands from 2854 to 2990 cm^{-1} was obtained in all liquefaction bio-oils, which is confirmed aliphatic functional groups present in the bio-oil. It may be obtained due to the decayed aromatic groups into the C-H groups or due to the presence of lipid compositions (Wang et al., 2019). High intensity of C-H group's absorbance band was found in case of PG and PH HTL bio-oil compared to that from PG-PH biomass mixture liquefaction bio-oil. Carbonyl (C=O) groups stretching vibration at 1672, 1669, 1710cm^{-1} in the bio-oil were found with higher intensity compared to the feed biomass indicates that higher carbonyl groups are present in the HTL bio-oil (Wang et al., 2019). These peaks are observed with higher intensity in the case of PG HTL bio-oil than PH and PG-PH mixture HTL bio-oil. Interestingly results were in goodconcurrence with the GC-MS analyses, with high ester content in the bio-oil obtained from HTL with alcoholic solvent (Hu et al., 2018). The absorption peak in between 1500-1620 cm^{-1}, confirmed that bio-oil hold of aromatics (C-C stretch) functional compounds. Higher intensity of this peak was observed inHTL of PG-PH mixture bio-oil compared to the individual PH and PG HTL bio-oils. C-H and C-O group absorbance peaks at 450 cm^{-1}, 1450cm^{-1} and 1203cm^{-1}, 1280 cm^{-1} were confirmed the ester functional group present in bio-oil. The bio-oil obtained with PG-PH liquefaction showed higher functional group peaks compared to the individual PH or PG biomass liquefaction bio-oil.

3.4 Analysis of organic carbon conversion into products

Total organic carbon (TOC) of the raw biomass and bio-chars were analyzed to understand the carbon conversion efficiency during the HTL reaction. The TOC of the bio-chars and TOC conversion into product are presented in Figure 5. It was observed that hydrothermal liquefaction of PH (69.8%) and PG-PH mixture biomass (61.2%) with water converted higher organic carbon products compared to the alcoholic solvent, while HTL of PG biomass with alcoholic solvent showed higher organic carbon conversion (57.3-58.2%) compared to the water as a solvent (52.2%). Overall the highest total organic conversion efficiency (69.8%) was foundfor PH biomass with water solvent. The less amount of organic carbon conversion observed, may be due to the bio-char reacting with the active volatiles (bio-oil) or the alcoholic methoxy/ethoxy group which enhanced carbon in bio-char and reduced the conversion. Whereas, the opposite trend has been obtained in the case of PG biomass as the alcoholic solvent increased the organic carbon conversion. In this case, the alcoholic solvent reduced the repolymerization and enhanced the degradation of

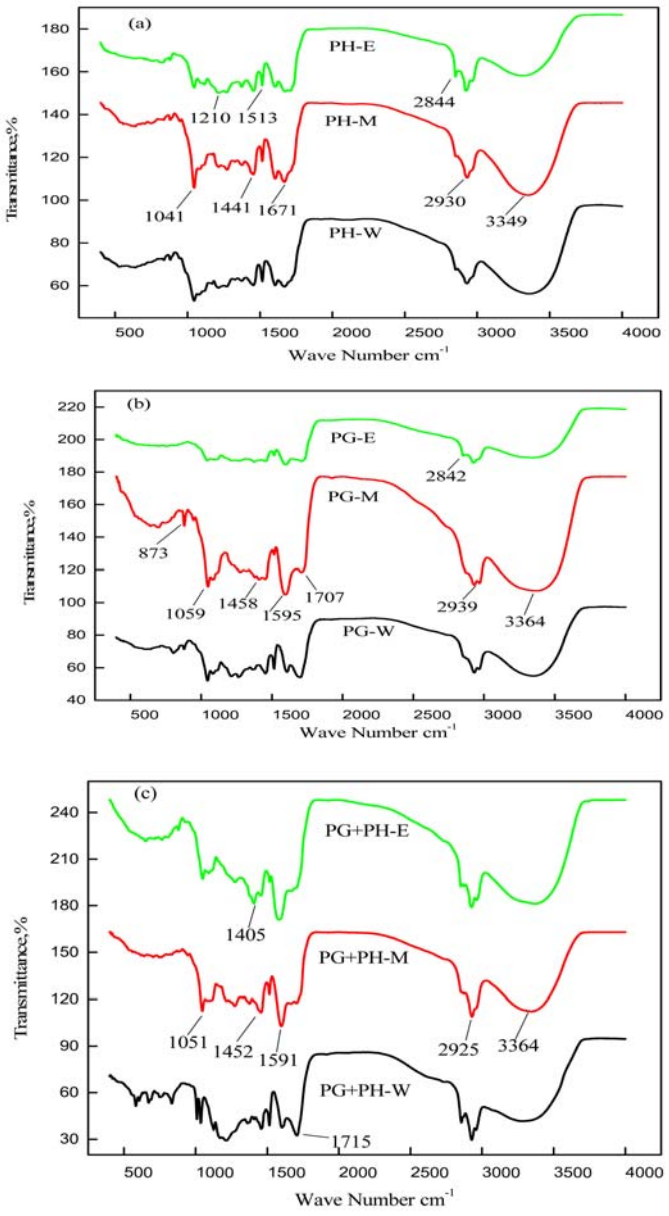

Figure 4. FT-IR analysis of bio-oil obtained from hydrothermal liquefaction (a) PH, (b) PG and (c) PG-PH mixture bio-oil.

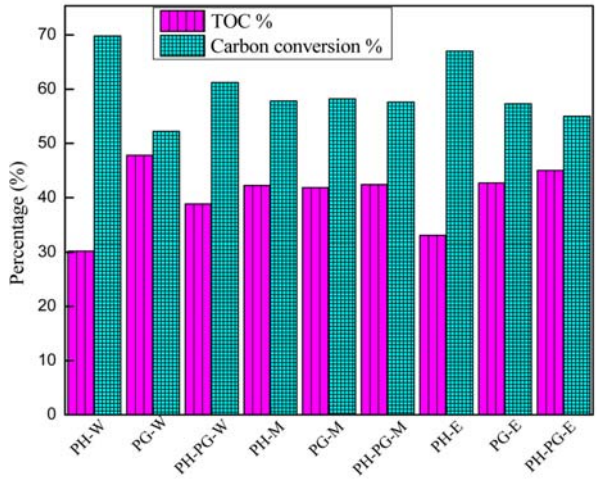

Figure 5.Comparison of Total organic carbon (TOC) conversion into product at optimal conditions.

biomass composition into the bio-oil/aqueous phase or gas products. Hence, it was observed that the reaction solvents as well as the biomass mixture influence the product yields. Further to understand the carbon utilization into the valuable product, TOC of the aqueous phase has been analyzed.

3.5 TOC and TN Analysis of aqueous phase

The aqueous phase is acknowledged as an important fraction for making the HTL process economically feasible due to the allocation of significant amounts of carbon. In table 5 total organic carbon (TOC) and total nitrogen (TN) analysis results are shown. Compared to the individual biomass PG and PH HTL reaction aqueous phases (6970 and 4194 mg/L), the mixture of PG-PH HTL aqueous phase showed significantly higher (57000 mg/L) total organic carbon (TOC). The higher TOC value in the PG-PH HTL aqueous phase could be due to the improved deprotonation of phenolic derivative compounds which leads to increase in their ionized form and thus became more soluble in water. The total nitrogen (TN) content highly depends on the protein composition present in the biomass (Wu et al., 2020; He et al., 2020). Higher value of TN was found in the case of PH HTL aqueous phase (276 mg/L) compared to the PG and PG-PH HTL aqueous phase (170 and 199 mg/L respectively). The nitrogen content obtained in the aqueous phase after the hydrothermal liquefaction, which is might be nitrogen containing aromatics compounds being protonated and becoming water soluble which decreased the nitrogen derivative compound in the bio-oil (Zhang et al., 2017). The lower TN was observed in the case of PG-PH HTL aqueous phase (199 mg/L) compared to PH HTL aqueous phase (276 mg/L). This is may be due to the carbohydrate present in biomass reacts with the produced ammonia and formed water insoluble nitrogen compounds via Maillard reaction (Chen et al., 2017; Zhang et al., 2015).

Table 5: Analysis of aqueous phase obtained from optimum conditions

Sample	TOC (mg/L)	TN (mg/L)
PH-300	4194	276
PG-300	6970	170
PH+PG-300	57000	199

3.6 Solid residue analysis

To understand the biomass functionality changesduring the HTL reaction, the obtained bio-chars have been analysisby the FT-IR characterization method (figure 6).The raw biomass spectra as discussed, earlier (Ayushi et al., 2019) showed several vibrations peaks corresponding to O-H, C-H, C=O, C=C, and C-O, C-C-O stretching due to the different composition presence in the biomass such as cellulose, protein, hemicellulose and lipid (Hu et al., 2018). The obtained bio-char showed lower intensity and new functional group vibrations peaks (3424, 2930, 1608 and 1035 cm^{-1}) in bio-chars. The decreased intensity of such functional groups could becleavage of C-H and C-O/C=O functional groups into gaseous molecule during the liquefaction reaction. The presence of peaks at 1608 cm^{-1} indicated to the C=C stretching vibration corresponding to the aromatic functional compounds (Han et al., 2019; Biswas et al., 2017). The higher intensitypeaks in PG-PH mixture bio-char indicated that they are rich in aromatics than individual PH and PG biomass HTL (Hu et al., 2018). C-H vibrations peak at 1460-1480 $cm^{-1}$was observed in PG and PG-PH biomass, while absorbance band was not observed for PH bio-char. The obtained bio-char showed higher intensity compared to the raw biomass feed, due to the amorphous components formed during the hydrothermal liquefaction. Surface morphology of the bio-chars were showed that the different compositions breakdown differently under different solvents, which makes surface morphology distinct from each other is shown in figure 7. The small

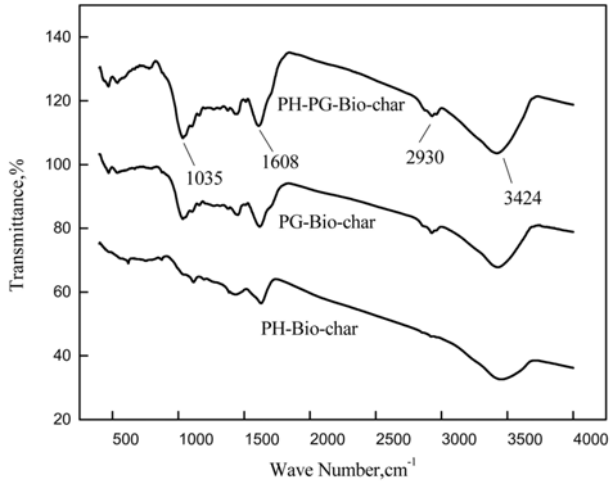

Figure 6.FT-IR analysis of bio-chars obtained from different reaction conditions.

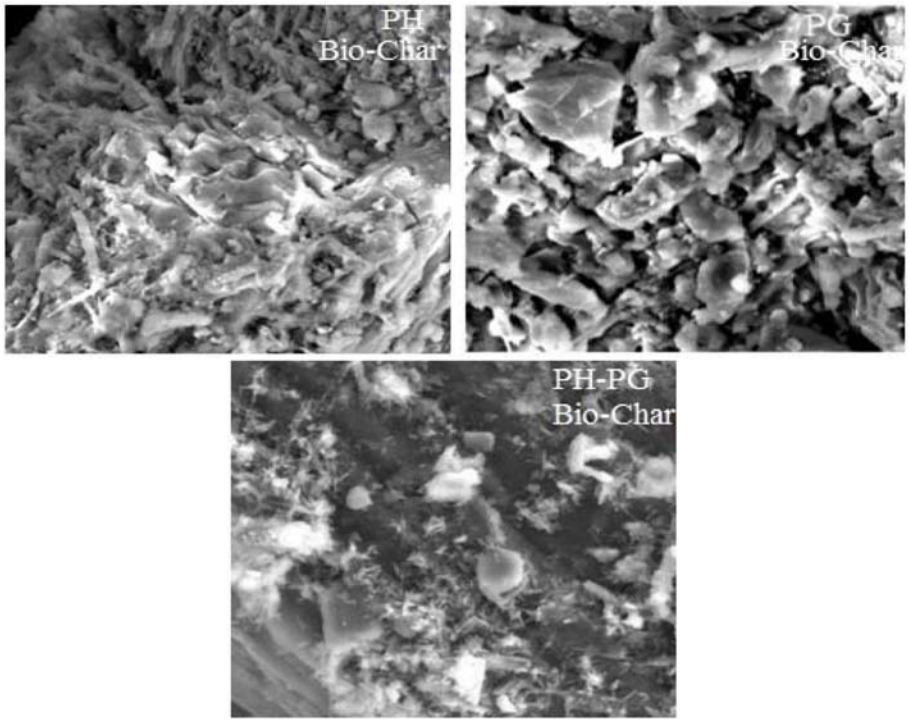

Figure 7.SEM analysis of Bio-char.

granules were observed in the case of individual and mixture of biomass HTL bio-chars which might be due to the high ash or inorganic metal existence in the bio-char surface.

4. Conclusions

Hydrothermal liquefaction is a promising method for the production of bio-fuels from the aquatic biomass. The maximum conversion of 98.4% was observed for individual PH biomass with water solvent. In case of water solvent, the maximum bio-oil was obtained for PG 13.82 wt.%, PH2.57 wt.% and a mixture of biomass (PG-PH) 8.84 wt.% at 300 °C. Alcoholic solvent such as methanol produced higher bio-oil (7.67 wt.%) with PG biomass than other solvent and biomass mixture. The GC-MS analyses showed that the mixture of PH and PG dominated the phenolic compounds while individual biomass liquefaction majorly content nitrogen containing and ester compounds. The higher intensity aromatic functional groups peak at 1500-1620 cm^{-1} observed in the case of HTL of PG-PH mixture bio-oil compared to the individual PH and PG HTL bio-oil. Compared to the reaction individual biomass PG and PH HTL aqueous phase (6970 and 4194 mg/L), the mixture of PG-PH HTL aqueous phase showed significantly higher (57000 mg/L) total organic carbon (TOC).

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