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HYDROTHERMAL LIQUEFACTION OF MICROALGAE CULTIVATED IN A PHOTOBIOREACTOR USING THE WASTEWATER EFFLUENT FROM AN ANAEROBIC REACTOR

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ABSTRACT

The rapidly increasing demand for energy depletes all fossil fuels available in the earth and searches for new alternative sustainable and renewable sources of energy. Among the different types of renewable sources of energy, microalgae are considered to be one of the potential feedstock for producing biofuels because of their primitive structure, high growth rate, carbon-neutral capacity and also its renewability. Recently, the researchers are focusing on several technologies that are capable of processing wet harvested microalgae to produce bioenergy in an easy way with using less energy and expenditure. Among the technologies, hydrothermal liquefaction (HTL) is one of the most alternative technology which producing liquid energy (biocrude) from wet microalgae by thermochemical conversion, next to gaseous, aqueous and solid by-products. The analysis of temperature parameter is presented here with constant holding time and without any addition of catalysts. The technology traditionally works at moderate temperatures (200^0-374^0C) and high pressures (2-20 MPa) although recent study is done at temperatures (260^0-280^0C) and pressure less than 5 Mpa with a holding time of 60 minutes. Biocrude obtained from a co-culture of microalgae (e.g. *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus simris002*) was cultivated in an 8-L photobioreactor using the wastewater effluent from an anaerobic reactor through this conversion process which is then further upgraded to biofuels. Finally, FTIR analysis was done to the biofuels for comparison. The FTIR data shows that the quality of biocrude heated at 280℃ is better than biocrude heated at 260℃.

Keywords: *Microalgae, Hydrothermal liquefaction, Biocrude, Biofuel, FTIR.*

1. INTRODUCTION

Wastewater has become a rising problem in the world which is now facing the third-generation revolution, but this wastewater can be an asset if we utilize it to make a profit as well as environment friendly. Wastewater can be treated in baffled reactor and then made it suitable for microalgae growth with proper aeration and sunlight. Then, the microalgae can be converted into bio-crude using thermochemical conversion (Suali & Sarbatly, 2012). There are mainly four types of thermochemical conversion which are transesterification, steam reforming, pyrolysis and hydrothermal liquefaction (HTL) (Goyal H.B. et al., 2008). Due to microalgae having high mass fraction of water (80%-90%), traditional thermochemical processes like pyrolysis and gasification are economically not very sustainable (Patil et al., 2008). The more sustainable process is the hydrothermal liquefaction whose primary requirement is water. Water in sub-critical state behaves like a catalyst and solvent for the HTL reaction (Patrick et al., 2001). One of the benefits of the process is that wet microalgae could be used in the HTL process where other conversion processes need dry microalgae. Other advantages are that HTL can be operated at moderate temperatures (200-374°C) and pressure (2-20 MPa) where pyrolysis requires higher temperature conditions (400-450°C) (Hu et al., 2018). The HTL is the low-cost process which has operational flexibility and at the same time enhances the capability of getting high yield with critical attributes such as lesser heteroatomic content, formation of low biochar and energy recovery. These are the factors for increased research interest towards it in recent years (Gollakota A.R.K. et al., 2018).

Hydrothermal liquefaction of microalgae is the thermochemical conversion which converts microalgae into high quality biofuels. The reactions occur in a hot environment under high pressure where water acts as a solvent to break down the solid biopolymeric structure to mainly liquid compounds (Toor S.S. et al., 2011). The three major steps of HTL are depolymerization, decomposition, and recombination (Toor S.S. et al., 2011). These reactions occur in the sub-critical state of water having some different characteristics. Water in this state can soluble organic compounds (hydrophobic). The ionic product of water (H^+ and OH^-) are available in the solution for acid and base catalyzed reactions which is in two orders of advanced magnitude (10^{-12} compared to 10^{-14} at 25°C). There are two main reactions in HTL: hydrolysis and recombination (Garcia Alba et al., 2011). In hydrolysis reaction, the decomposition and depolymerization of the microalgae breaks them into small compounds. These small compounds are highly reactive. High pressure and temperature enhances the polymerizing of the small compounds to form bio-crude, gas, and solid compounds (Demirbaş, 2000). With the increase of holding time, the viscosity of bio-crude decreases (Minowa T. et al., 1995). The gaseous fraction like CO_2, H_2, CH_4, N_2, C_2H_4 and C_2H_6 presents a yield of approximately 20% of the original organics existing in the microalgae feedstock (Brown et al., 2010).

Biofuels as a potential substitute for fossil fuels are a promising renewable energy source. Moreover it alleviates environmental complications such as global warming, climate change, etc. (Hill J. et al., 2006). Unlike other sources like solar energy and wind power, biofuels are carbon substances that can directly substitute petroleum products like transportation fuels (Schenk et al., 2008). If biomass were grown for energy to an amount equal to that consumed during any given production period, there would be no net increase of CO_2 in the atmosphere (Gao & McKinley, 1994). At present, biomass has the ability to fulfill 30% of global fuel demand through the conversion process to intermediate solid, liquid or gaseous biofuels without affecting the food production as well as environment. Microalgae are microscopic photosynthetic organisms that can absorb more CO_2 due to its higher photosynthetic efficiency, faster growth rate and higher area-specific yield than terrestrial biomass (Aresta M. et al., 2005). Microalgae needs water, light, carbon source and nutrients to grow and their main constituents are protein, carbohydrates, and lipids. Recently, another fraction named algaenans in the form of long aliphatic chains that is found in the outer cells of microalgae is another source of biofuels (Garcia Alba et al., 2011). The algaenans which are insoluble and non-hydrolysable biomacromolecules, resistant to drastic chemical treatments (Gelin et al., 1999).

Cultivation of microalgae brings along some advantages (Mata T.M., 2010) as they use the nutrients like NH_4^+, NO_3^- or PO_4^{3-} to grow. As wastewater can supply these nutrients, wastewater is suggested to

cultivate microalgae and at the same time bioenergy is being produced. As the HABR effluent water holds large amount of nitrogen and phosphorus content, it is suitable for microalgal growth.

During the past decades, microalgal research has been focused mainly on optimization of cultivation methods, but less effort was spent on their processing for biofuels, chemicals or energy production. The main objective of this research is to analyze the quality of biocrude processed in low temperature (260°C and 280°C) and pressure less than 5 Mpa based on FTIR spectrum analysis. The IR analysis shows the chemical compounds present in the biomass, biocrude and biochar. Thus, biocrude quality assessment is done. The conversion process extracts high NO_x emissions, due to the high amounts of nitrogen in chlorophyll and proteins, very abundant in microalgae cells (Costa & De Morais, 2011). Another drawback of this process is that the aqueous phase contains substances toxic to several organisms and it can be classified as a petrochemical refinery wastewater (Appleford et al., 2005). Recent researches detect that several nitrogenous organic compounds were identified. In conclusion, the authors stated that HTL aqueous phase should be treated before discharging it to the environment.

2. METHODOLOGY

2.1 HABR Effluent

The wastewater was accrued from the septic tank. The water was then pumped to the HABR at 121 mL/hr. and the water passed through the baffle chambers to purify the wastewater. The reactor has seven chambers where the last two chambers are filter media. The individual chamber was again divided into two portions by hanging baffles, which separated each chamber in down- and up-flow zone. The ratio between down-flow and up-flow was 1:4, and the lowest portion of the baffle was inclined at 45° (Hasan M. et al., 2018). The water is forced into the reactor using pump for 10 minutes every hour. The water is suitable for microalgae growth as it was observed that the wall of filter media of HABR is covered by greenish color of microalgae. The water will also be used in HTL reactor as water behaves as the solvent for hydrothermal process at temperature 200~300°C. Water, temperature over 200°C and pressure over 2 Mpa, stays in sub-critical state. Normally water doesn't soluble the organic contents but in subcritical state water reacts with organic molecules. Then organic chemical reactions like depolymerization, decomposition, recombination of microalgae take place. Here water causes organic matter to break down and reorganize into fragments that is transformed into hydrocarbons. Thus, water helps to convert directly biomass to biocrude in HTL process. The main reason to use HABR effluent in microalgae cultivation is that the water contains a large amount of nitrogen and phosphorus content which supplies essential nutrients for microalgae growth.

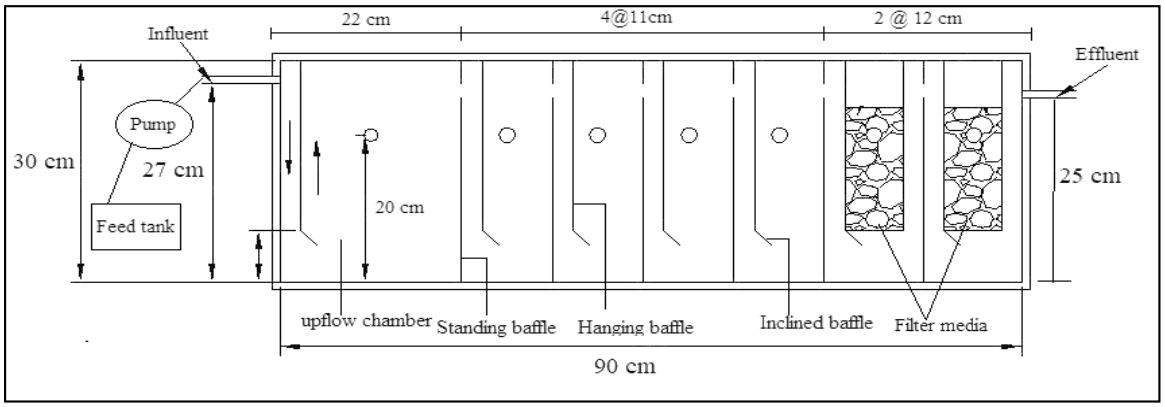

Figure 1: The schematic diagram of HABR

2.2 Microalgae Cultivation

A microalgal co-culture of *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus simris002* (Figure 2) was cultivated in plastic containers (8000cc) and the plastic used was colorless. The containers had HABR effluents where 20ml microalgal co-culture seeds were poured into the container. The aeration was done for 12 hours (10 minutes per hour) per day for 5 days. Then by coagulation and flocculation, the microalgae were separated and collected for HTL process (Figure 2).

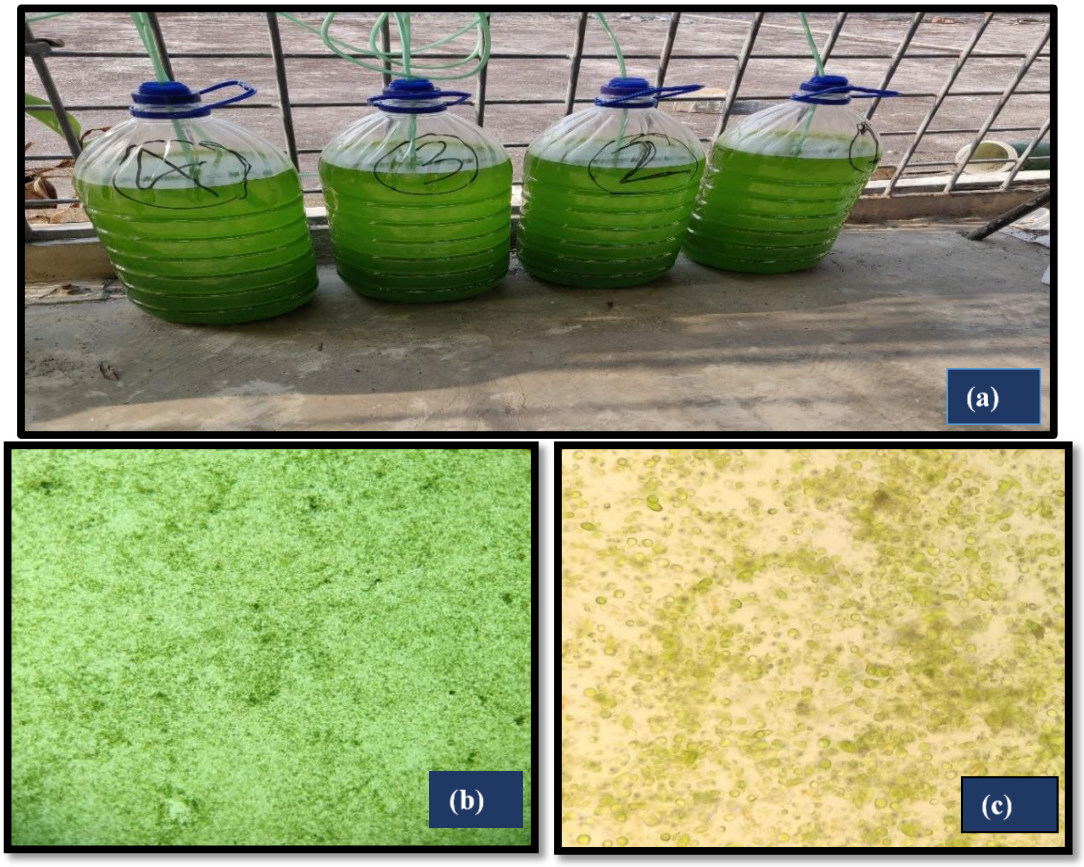

Figure 2 : Microscopic view of microalgae co-culture (a) cultivation (b)10X magnification and (c) 40X magnification

The microalgal co-culture was selected because of its high lipid content, high growth rate and less pollution (Chen et al., 2014). The classification of microalgae was done by matching its shape in the microscope.

2.3 Coagulant, Coagulation, and Flocculation

Recovery of microalgal biomass could be done using low-cost bio-coagulant. There may be chemical coagulants which are used to coagulate microalgae but they are very costly and have adverse health effect. So, the natural coagulant *Moringa oleifera* was used to flocculate the microalgae and let it settle. The main reasons for the selection of this bio-coagulant are locally available, cheap, non-toxic and environment friendly. The coagulation and flocculation were done by adding 50mg coagulant and the stirrer speed was set to be 100 rpm and the settling time was 30 minutes (Figure-3).

2.4 Dichloromethane

Dichloromethane (DCM) was bought from NIP Chemicals and Pharmaceuticals Ltd. with high purities (> 99.8wt%). Dichloromethane was used for solvent extraction which extracts the bio-crude from the product mixture. Moreover, DCM is dense, non-polar and highly volatile which makes homogeneous solutions with most of the organic solvents. DCM increases the solubilization of the bio-crude as well as extract maximum bio-crude from the product mixture (Chopra et al., 2019).

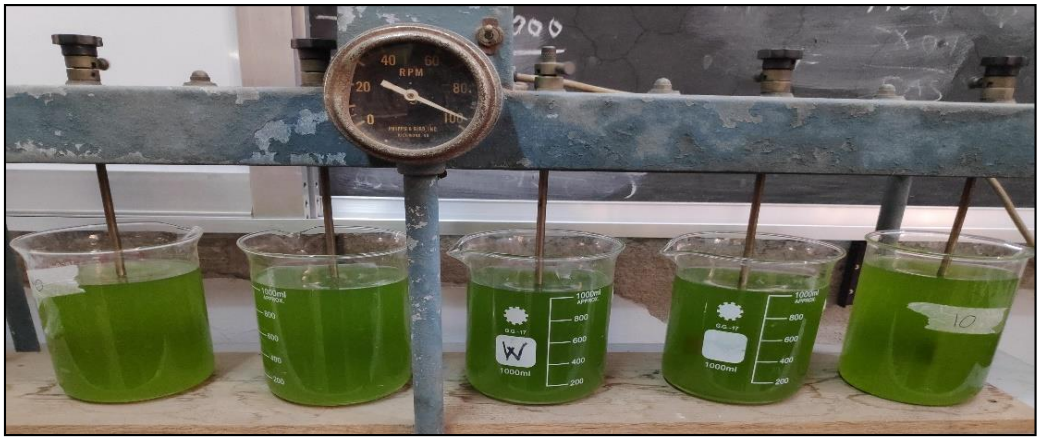

Figure 3 : Coagulation and flocculation process

2.5 HTL Reactor Setup

Reactions were carried out in a hydrothermal synthesis reactor (25ml) made of stainless steel 316 grade and which has a capacity of the working pressure of 5 MPa. The container specified as ppl liner of 25 mL was used to hold the microalgal solution and the reaction occurs inside the liner in high pressure and temperature. The liner has the capacity to be stable up to temperature 280°C and pressure of 5 MPa. The reactor has uniform wall thickness which allows the uniform heating of the reactor. There is also a rod attached to the top portion so that the reactor could be tightened firmly (Figure 4).

Figure 4 : Hydrothermal synthesis reactor

2.6 Experimental Methodology

In each experimental run 15 ml of sample including microalgae and HABR effluent water was taken into the reactor. The microalgae co-culture was taken 10wt% measured by the moisture content of the sample (Anastasakis & Ross, 2011). The reactor loading was 50% (Anastasakis & Ross, 2015). Then the reactor was taken to the muffle furnace and heated to the desired temperature (260°C, 280°C) (Tommaso et al., 2015) at an average heating rate of 60°C/min. After reaching the desired temperature

the sample was kept inside the reactor for reaction time of 60 minutes (Ross et al., 2010). Once the holding time was finished the reactor was taken out from the muffle furnace and cooled it down by water bath in a basin for 5 minutes and 60 minutes at room temperature.

The outcomes of HTL of microalgae included gases, solids, aqueous phase, and bio-crude (Figure 5). After cooling down the reactor was cautiously opened and mixed with 30ml dichloromethane (DCM). The main reason for adding DCM is to extract the organic components from both liquid and solid products and also to be miscible with organic solvents. The bio-crude is also defined as DCM-soluble fraction (Biller & Ross, 2011). They were then taken into a conical tube and centrifuged at 3000 rpm for 10 minutes (He et al., 2018). After centrifugation, the organic phase dissolved in dichloromethane stays at the bottom and the aqueous phase stays at the top and the biochar stays in the middle that separates the organic phase with aqueous phase (Figure 6). Then the aqueous phase was carried into a glass tube by a pipette, and the DCM phase (dissolving biocrude) was taken out through filtering and then moved into a pre-weighed glass tube, and then dried at 40°C for 1 hr. The solids remaining on filter paper were dried at 100°C for at least 24 h before weighed (He et al., 2018). Two independent runs were carried out by the above procedure. Then FTIR analysis was done to the biocrude samples to represent the quality of biocrude samples.

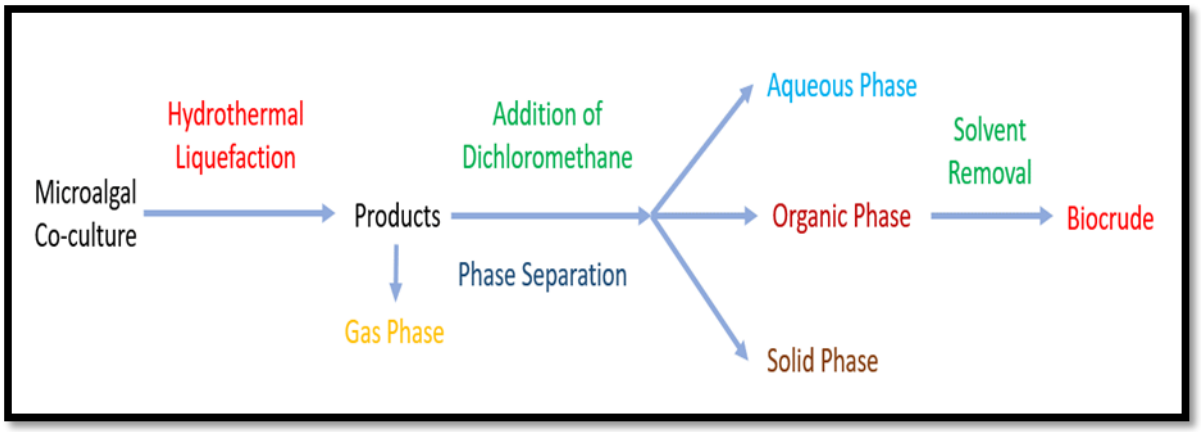

Figure 5 : Procedure for reaction and product work-up

2.6 FTIR Analysis

The characterization and elucidation of the functional group present in dry microalgae, biocrude and biochar were determined using FTIR spectroscopy study (Mahapatra & Ramachandra, 2013). FTIR analyses were conducted on microalgae dry cell, biocrude and biochar at room temperature using Shimadzu (IRTracer-100) FTIR spectrophotometer (Ansari A.A. et al., 2017). The dried algal biomass and biochar samples were further broken into powder as the requirement of the instrument. The powder of the samples was pressed against the diamond cell prior to scanning. The extracts from these samples were observed for their functionalities in the spectrogram. The spectra were collected in the mid-IR range from 4000 to 800 cm^{-1} (at a spectral resolution of 2 cm^{-1}) and data were analyzed using Microsoft Excel, irAnalyze-RAMalyze (Lab-Cognition GmbH & Co. KG) and Essential FTIR (Operant LLC).

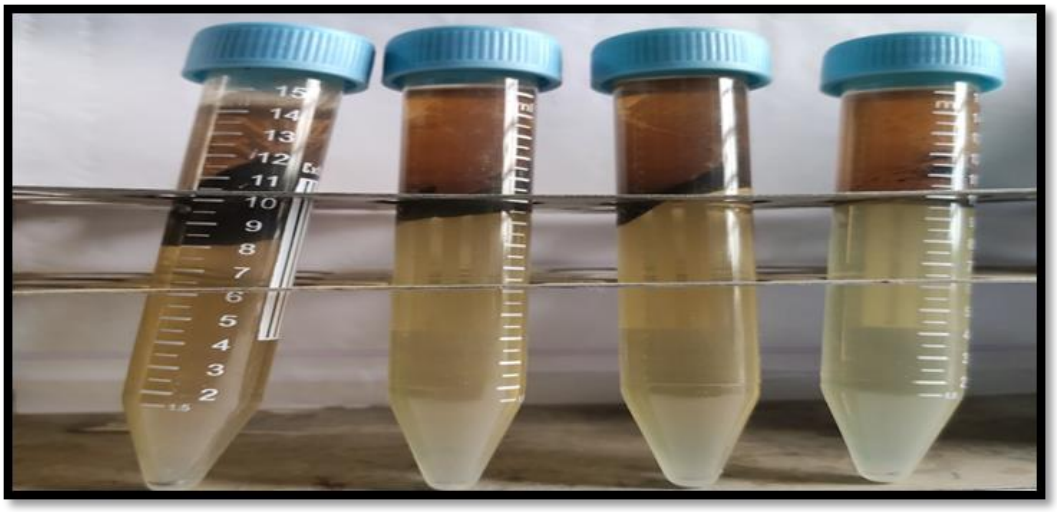

Figure 6 : Phase separation of HTL products

3. RESULTS AND DISCUSSIONS

3.1 Fourier Transformed Infrared Spectroscopy (FTIR) Analysis

FTIR Spectroscopy was employed to determine the presence of vibrations (stretching and bending) active functional groups (including CH_3 stretch, C=C-H, C-C-COOH (C=O), N-H Deformation, C-CX-COOH (C-O), C-H (Aromatics), C-H Stretch (Alkyl), C=C-COOH (O-H), N=O, C-N Stretch, N-H Stretch, C-N=O, N-H Bend, C-N-H Bend, Si-O Stretch) in dry microalgae co-culture cell sample, biocrude and biochar as presented in Table 1, Table 2 and Table 3 (Ansari et al., 2017). The most characteristic IR spectra peaks of the microalgae dry cell samples are shown in Figure 7, Figure 8 and Figure 9. IR spectra for all samples were found to be contaminated for CO_2 contamination (650-700 cm^{-1} and 2250-2450 cm^{-1}) and water vapor (3440- 3950 cm^{-1}). Removing these contaminations, baseline correction was done in software and then the spectrums were analyzed for active functional groups.

3.1.1 Dry Microalgal Biomass

The main absorbance bands of dry microalgal co-culture cell that reveals the specific functional groups and the presence of a related class of compounds was discussed (Figure 7 and Table 1). The band at 1406 cm^{-1} is related to methylene CH_2 bending and methyl CH_3 bending. The bend at 879 cm^{-1} may be due to the presence of aromatic compounds. Some small bands in the range (3010-3100cm^{-1}) shows the presence of double bonded carbon compounds (C=C-H). The strong peak at 1033 cm^{-1} is attributed to either C-O stretching in carbohydrates or Si-O stretching which is present in the cell wall of microalgae (Si-O-C), which disappeared in biocrude and biochar samples (Figure 8 and Figure 9). Peaks at 1537 cm^{-1} and 1645 cm^{-1} indicate the N=O bend, amide (C-N=O) bends, N-H bends, C-N-H bend which belongs to the protein fraction. The peak at 1718 cm^{-1} represents C=O which is rather weak according to the absorption of other peaks.

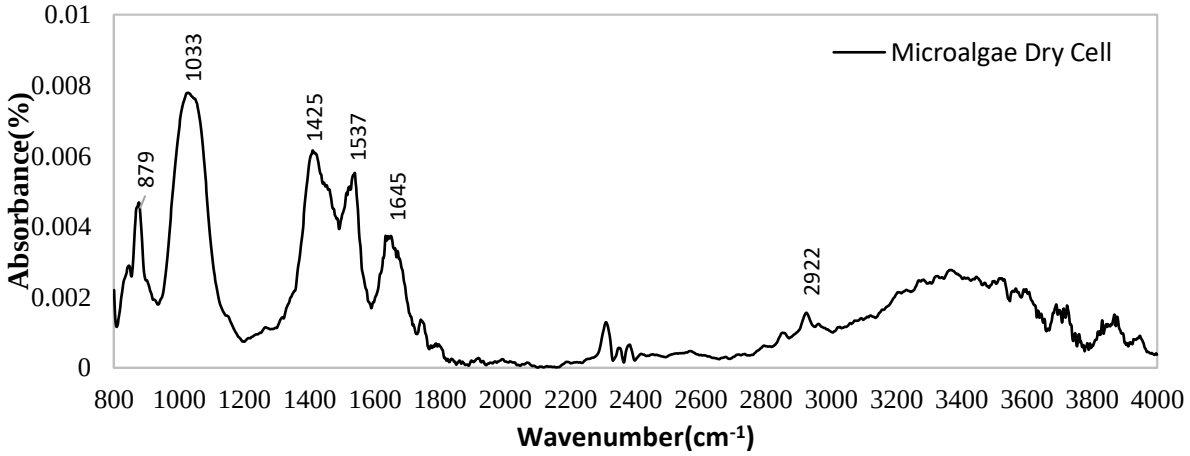

Figure 7: FTIR spectrum of microalgal co-culture dry cell

Table 1: FTIR band assignments for microalgae dry cell

Functional Groups	Spectra range (cm^{-1})	Strength of Spectra ranges Microalgae dry cell
CH3 / CH2 stretch	1400-1450	Strong
C=C-H	3010-3100	Medium
C-C-COOH (C=O)	1690-1715	Weak
C-H (Aromatics)	860-900	Strong
N=O	1500-1580	Stronger
C-N=O Amide	1630-1685	Strong
C-N-H Bend	1525-1550	Strong
Si-O/C-O Stretch	950-1050	Strong

3.1.2 Biochar

FTIR analysis of biochar samples biochar HTL at 260℃ (biochar 260) and biochar HTL at 280℃ (biochar 280), which were HTL effluent's solid phase, were observed to have quiet similar peaks (Figure 8). The aromatic ring skeletal bending (845-925 cm^{-1}) is found in both samples. The C-CO bend (1000-1150) is observed in both samples but the biochar 280 has more intensity according to absorbance. The both biochar samples have strong peak at 1410-1415 cm^{-1} which denotes the presence of S=O bend. The both samples have peaks at the range 1500-1580 cm^{-1} and 1630-1660 cm^{-1} which represents the N=0 and C=C bending of p-nitrophenol compound. A peak of 1848 cm^{-1} is observed in biochar 260 sample which represents C=O bending which is not seen in biochar 280 sample. Some peaks of low absorbance were observed near 2900-2975 cm^{-1} which indicates C-H bending of carbohydrates.

Table 2 : FTIR band assignments for biochar samples

Functional Groups	Spectra range (cm^{-1})	Strength of Spectra ranges	
		Biochar 260^0C	**Biochar 280^0C**
C-H Stretch	2900-2975	Variable	Variable
C-O	1070-1100	Strong	Strong
N=O	1500-1580	Stronger	Stronger
C=C	1630-1660	Strong	Medium
C-CO	1000-1150	Strong	Strong
S=0 stretch	1340-1420	Strong	Strong
Ring Skeletal	845-925	Variable	Variable
C=O	1840-1850	Weak	-

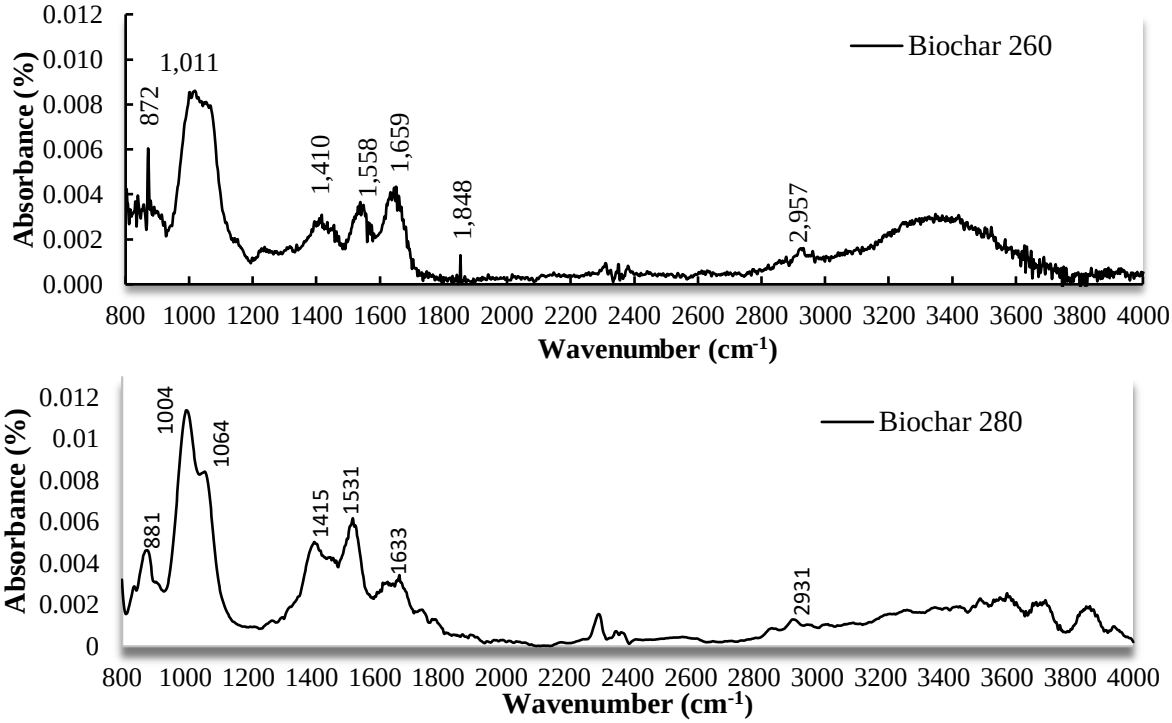

Figure 8: FTIR spectra of biochar at 260℃ and 280℃

3.1.3 Biocrude

IR spectra of biocrude HTL at 260℃ (biocrude 260) and HTL at 280℃ (biocrude 280)) were recorded between 800-4000 cm-1. The absorption bands that dominate the spectra of biocrude are aliphatic C-H bonds and organic groups containing oxygen, sulfur, nitrogen, and aromatics. The FTIR spectrum of the biocrude samples are presented in figure 9 and band assignments are shown in table 3. The broadband seen at 3445-3900 cm-1 for microalgal biomass can be attributed to O-H stretching which is not seen in the biocrude spectrums so this indicates the absence of moisture. The sharp peaks at 2859 cm^{-1}, 2855 cm^{-1}, 2920 cm^{-1}, 2924 cm^{-1}, 2957 cm^{-1} indicates the presence of methylene ($(CH_2)_4$-C) and alkyl group. Here the peaks in biocrude 280 samples are sharper and the intensity according to absorbance is more than biocrude 260 samples. The biocrude 260 sample has small peaks in 1734 cm^{-1} which indicates the presence of esters (COO), ketones aldehydes where no peaks were observed in biocrude 280 samples within this range. There was another strong peak of 1260 cm^{-1} indicates the presence of methyl (CH_3) group in biocrude 260 samples wherein biocrude 280 samples the peak is very weak. The peak in 1013 cm^{-1} in biocrude 260 sample defines the presence of C-O bend but this Bend is disappeared in biocrude 280 sample which is evidence of the hydrolysis reaction. Both of the spectrum has peaks around 1080 cm^{-1} which indicates the C-O-C bend but the intensity of absorbance is lower for biocrude 280 samples. Peaks at 1460, 1549, 1676 cm^{-1} indicate the methylene group, amide (N=O) and double bonded carbon (C=C) which also determines the presence of p-nitrophenol in biocrude 280 samples but no definite peaks were observed for biocrude 260 samples. The biocrude samples show less intense peaks for amide bonds and nitro groups as compared to biochar. This implies the majority of the nitrogen in the biomass has been captured in biochar.

Table 3 : FTIR band assignments for biocrude samples

Functional Groups	Spectra range (cm-1)	Strength of Spectra ranges	
		Biocrude 2600C	Biocrude 2800C
(CH2)4-C	2916-2936	Medium	Medium
C-H Stretch, Alkyl	2845-2975	Variable	Variable
CH2/CH3 Stretch	1415-1475	Variable	Variable
CH3 Stretch	1260	Very Strong	Weak
COO(Esters)	1720-1770	Medium	-
C-O-C	1080-1135	Strong	Strong
N=O	1500-1580	-	Variable
C=C	1610-1680	-	Variable
C-O Stretch	950-1050	Strong	-

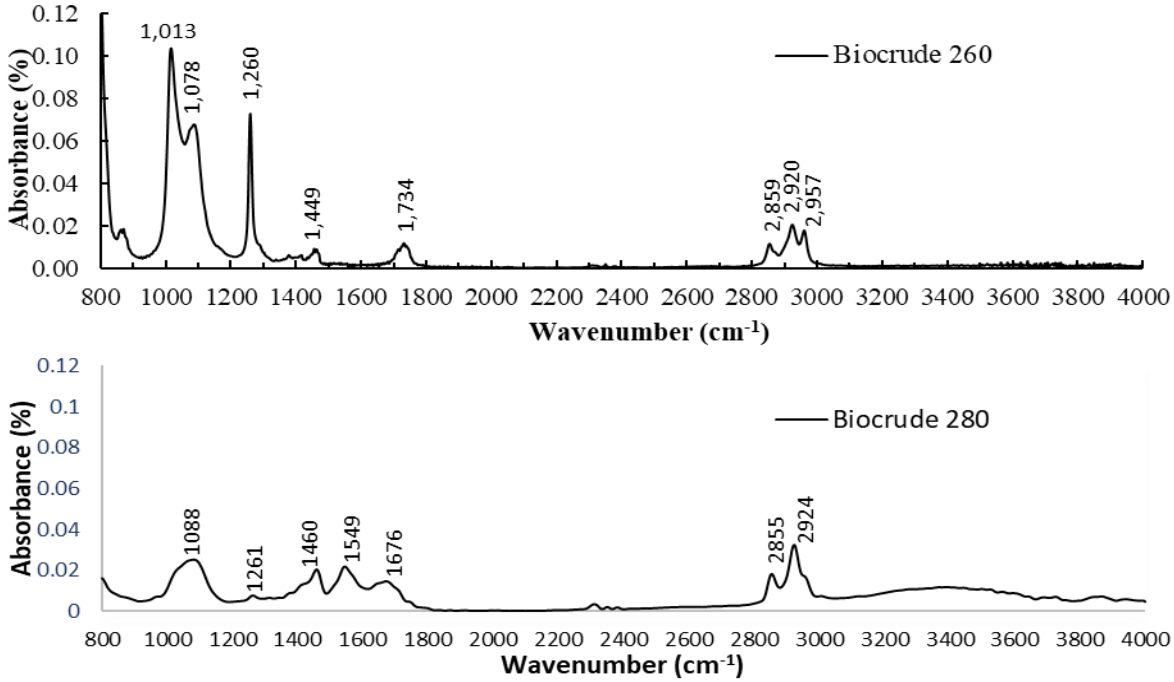

Figure 9: FTIR spectra of biocrude sample at 260℃ and 280℃

4. CONCLUSIONS

In conclusion, the analysis of biocrude FTIR clarifies the dehydration and decarboxylation reactions that happened as the C-H intensity rose. Again, the decrease in C-O and C=O peaks proved the decomposition step of hydrothermal liquefaction happened in the biomass. The thermochemical conversion of biomass to biocrude was proven. Between the two temperature steps of 260℃ and 280℃, the analysis of FTIR showed the biocrude 280 sample quality was better as the peaks for C-H had higher intensity level. The FTIR of both biocrude samples proved the reduction of nitrogen concentration compared to algal biomass. The nitrogen content in algal biomass shows that the microalgae absorbed the nitrogen substances from HABR effluent water. So, the microalgae are also treating the effluent water by decreasing its nitrogen content to reduce the drawback of HABR system. Furthermore, the microalgae also absorb CO_2 from environment to produce organic compounds using sunlight and photosynthesis process. The microalgae are the environment friendly micro-organism which can be cultivated in anaerobic reactor effluent using photoreactor and then converted into biocrude representing the alternate source for bioenergy.

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