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CHARACTERIZATION OF SALVINIA MOLESTA AND CHLORELLA PYRENOIDOSA FOR BIOFUEL APPLICATIONS USING FTIR AND TGA

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ABSTRACT

Microalgae and aquatic weeds are considered as promising feedstock for biofuel production due to its higher biomass productivity and lipid content. The characterization of the feedstock is important for biofuel production. In this work, an attempt was made for the characterization of *Salvinia molesta* and *Chlorella pyrenoidosa* using Fourier Transform Infrared (FTIR) and thermogravimetric analysis for biofuel production. The dried and grounded *S. molesta* and *C. pyrenoidosa* was used for both analyses. The FTIR spectra was recorded from wavenumber 400-4000cm^{-1}. The TGA was performed from 28-750^{o}C with a heating rate of 10^{o}C/min using powdered *S. molesta* and *C.pyrenoiodosa*. The FTIR spectra showed that lipid, carbohydrate, cellulose and fatty acids bands are predominant which indicates that *S. molesta* and *C. pyrenoidosa* can be used a potential feedstock for biofuel production. The thermogravimteric analysis showed the presence of three distinct stages such as drying, devolatilization and steady decomposition of heavy components such as lignin.

Keywords- Biomass productivity, FTIR, TGA, Lipid, Carbohydrate. *Salvinia molesta, Chlorella pyrenoidosa*.

I. INTRODUCTION

The biofuel production from microalgae like *Chlorella pyrenoidosa* and aquatic weed like *Salvinia molesta* is promising due to its higher areal productivity and lipid content (Mubarak et al., 2015) The major problems with *Salvinia molesta* are cloging of hydro-electric dams, restrict irrigation, causes flooding and erosion, reduce suitable habitat for native fish, such as eel and whitebait and it make the water unsuitable for drinking purposes (Mubarak et al., 2016a, Mubarak et al. 2016b). The characterization of microalgae and freshwater weeds are important to find its biochemical composition, oil or lipid content for biofuel applications. The lipid content of microalgae and aquatic weeds is the major concern for biodiesel production. There are different methods such as thin layer chromatography, Nile red screening method, mass spectroscopy to characterize the biochemical composition of micro-algae and aquatic weeds. However, these methods are rely on sample preparation step and are time consuming (Laurens and Wolfrum 2010). The use of FTIR spectroscopy for the characterization of algae and aquatic weeds reduces the problem of sample preparation and time. Fourier Transform Infrared (FTIR) analysis is method of analysis which uses intact cells with an involvement of measurement if infrared absorption in relation to a range of molecular vibrational modes (Meng et al. 2014). A particular molecular group can identify by their absorption bands, which allows macromolecules such as proteins, lipids, carbohydrates and nucleic acid (Dean et al., 2010). A few research works were reported with FTIR analysis with microalgae. Dilek et al., (2012) identified the spectral features of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* and reported 11 bands of peaks such as cellulose, lipid, protein, nucleic acid and carbohydrate. Laurens and Wolfrum (2010) studied the feasbility of characterization of four species of algae, *Nannochloropsis* sp., *Chlorococcum* sp, *Spirulina* sp and an unknown diatom using FTIR spectra. Another study reported by Sudhakar and Premalatha (2015) used FTIR, TGA and CHN analysis for the characterization of *Scenedesmus* sp. They reported that FTIR analysis clearly indicated the presence of lipid groups, alcoholic groups and carboxyl groups.

The TGA is used find the proximate biochemical compositions of biomass based on measurements of weight loss of biomass with temperature (Figueora et al., 2015). The weight loss with temperature is due to the evaporation of residual moisture or solvent and decomposition of organic matter (Bi and He 2013, Ferreora et al. 2015). The TGA can provide data for decomposition of organic and inorganic compounds, for identification of composition of the mixture, reaction-kinetic studies, the gravimetric precipitates can be evaluated, oxidative and reduction stability of a compound, the moisture, volatile and ash contents of the sample (Zabaniotou et al., 2008). The different appli-

cations of TGA analysis of microalgae are ash content determination, to study thermal characteristics of biomass and its components under a controlled environment and is often associated with kinetic modeling (Tang et al., 2011, Liu et al., 2015, Gai et al., 2014).

To the best of our knowledge, this is the first work reporting with FTIR and TGA analysis of *C. pyrenoidosa* and *S.molesta*. This study attempts to find the biochemical composition of *C. pyrenoidosa* and *S. molesta* using FTIR and thermogravimetric analysis.

II. MATERIALS AND METHODS

(i) *Collection and processing of S. molesta:* The *S. molesta* was collected from fresh-water bodies nearby Mavoor, Calicut, Kerala, India and washed thoroughly with tap water to remove impurities such as sand and mud. The thoroughly washed *S.molesta* is dried in sunlight and grounded using a mechanical pulverizer to make a size less than 1 mm.

(ii)*Cultivation of C.pyrenoidosa:* Microalgae, *Chlorella pyrenoidosa* was procured from National Centre for Industrial Microorganisms (NCIM), Pune, India. The stock culture of *Chlorella pyrenoidosa* was grown in Bold Basal Media (BBM) under room temperature with sunlight. Each litre of BBM contained KNO_3-0.25g, K_2HPO_4-0.074g, $MgSO_4 \cdot 7H_2O$-0.073g, $CaCl_2 \cdot 2H_2O$-0.024g, NaCl-0.025g, $FeSO_4$-0.005g, Na_2EDTA-0.045g and the pH was adjusted to 7. The preparation of stock culture was done by adding 10% inoculum to the sterilized BBM (Mubarak et al. 2018).

(iii) ***Harvesting and processing of C. pyrenoidosa****:* The *C. pyrenoidosa* cultivated was harvested using centrifugation on 16th day. The *C. pyrenoidosa* culture was taken in 50 ml capacity plastic tubes and centrifuged at 4000 rpm for 10 min. The upper part of water was discarded and *C. pyrenoidosa* pellet is dried in hot air oven at 50^{o}C for 3 h. The dried *C. pyrenoidosa* was grounded in mortar and pestle to form a size less than 1 mm (Mubarak 2016)

(iv) ***FTIR analysis of S.molesta and C. pyrenoidosa:*** The analysis was carried out in Nicolet FTIR spectrometer (Madison, USA). The instrument was equipped with a mercury cadmium telluride (MCT) detector and spectra were recorded from 400-4000cm^{-1} at a resolution of 4cm^{-1}. About 1.5mg of *C. pyrenoidosa* and *S. molesta* were ground with 100mg of spectroscopic pota-ssium bromide (KBr) powder in a mortar and pestle was used for the analysis. About 10-12tonnes of pressure were applied to this mixture for 5 min to obtain 1 mm transparent pellets (Sudhakar and Premalatha 2015).

(v) *Relative contents of lipid and carbohydrate:* Amide I with wave number ranges 1584-1724 cm^{-1} was chosen as internal reference peak for relative assessment of lipid and carbohydrate (Meng, et al., 2014). The absorption area of amide I band is calculated using the FTIR data using Excel 2010. The lipid band is within the wave number of 2800-3000 cm^{-1} and the area of this band is calculated. The relative lipid content was estimated by using the ratio of lipid band area to the amide I band. The area of carbohydrate band (950-1200 cm^{-1}) was calculated using FTIR data using Excel 2010. The relative carbohydrate content was estimated using the ratio of area of carbohydrate band to the amide I band (Sudhakar and Premalatha 2015).

(vi)Thermogravimetric analysis of S. molesta and C. pyrenoidosa: The TGA data were measured with a diamond model TGA (Perkin, Elmer, USA) in a nitrogen atmosphere. 7mg of *C. pyrenoidosa* and *S. molesta* were taken in a ceramic crucible and heated from room temperature to 750^{o}C at a rate 10^{o}C min^{-1} using air as medium under static condition (Sudhakar and Premalatha 2015).

III. RESULTS AND DISCUSSION

The characterization of *S.molesta* and *C. pyrenoidosa* was done using FTIR and thermogravimteric analysis. The FTIR spectra with wavenumber range of 400-4000 cm-1 was plotted and thermogravimteric analysis was done with temperature range of 28-750^{o}C with a heating rate of 10^{o}C/min.

(i) ***FTIR analysis of C. pyrenoidosa and S. molesta:*** As shown in Table 1, the FTIR analysis provides about 11 distinct bands with different wave number. It was clear from the data shown that, lipid- carbohydrate band is present in both

Table 1: Assignment of bands found in FTIR spectra of *S.molesta* and *C.pyrenoidosa*

Band	Band assignment	Wave number cm^{-1}	Functional groups	Present study	
				S. molesta cm^{-1}	*C.pyrenoidosa* cm^{-1}
1	$_{v}$(O-H) / $_{v}$(N-H)	3400-3200	Water, protien	Not identified	Not identified
2	$_{vas}CH_3$	2960	CH_3 methyl group	2969.84	2973.70
3	$_{vas}CH_2$	2930	CH_2 methyl group	Not identified	Not identified
4	$_{v}CH_2$, $_{v}CH_3$	2850	CH_2 and CH_3 methyl and methylene group	1586.16	Not identified
5	$_{v}$C=O	1745	Ester of lipids and fatty acids	1733.69	1737.55
6	$_{v}$C=O	1655	Protein (Amide I)	1438.64	1462.74
7	$_{\delta}$N-N, $_{v}$C-N	1545	Protein (Amide II)	Not identified	Not identified
8	$_{\delta as}CH_2$, $_{\delta as}CH_3$	1455	CH_2 and CH_3 methyl and methylene groups	1349.93	1213.01
9	$_{\delta s}CH_2$, $CH_3/_{\delta}$C-O	1390	CH_2 and CH_3 of protiens/ carboxylic groups	1169.62	Not identified
10	$_{vas}$P=O	1240	Phosphodiester of nucliec acids and phospholipids	1109.83	Not identified
11	$_{v}$C-O-C	1200-900	Polysaccharides/siloxane	914.09	1048.12
12	$_{v}$Si-O	1075 and 950	Siloxane, silicate frusters	Not identified	Not identified
13	P-O-P	980-940	Polyphosphate	Not identified	Not identified

$_{v}$= symmetric stretching, $_{vas}$= Assymmetrical stretching, $_{\delta}$= symmetric deformation (bend), $_{\delta as}$= Assymmetric deformation (bend)

S. molesta and *C. pyrenoidosa*. Also, cellulose-fatty acids, carbohydrates bands are predominant in *S. molesta* and *C. pyrenoidosa*. Hence, it can be concluded that the *S. molesta* and *C. pyrenoid-dosa* are potential feedstock for biofuel production. The relative lipid ratio, carbohydrate ratio for *S. molesta* are 1.498, 1.797 respectively. The same values are obtained with *C. pyrenoidosa* which indicates the biochemical compositions of *S. molesta* and *C. pyrenoiodosa* are similar.

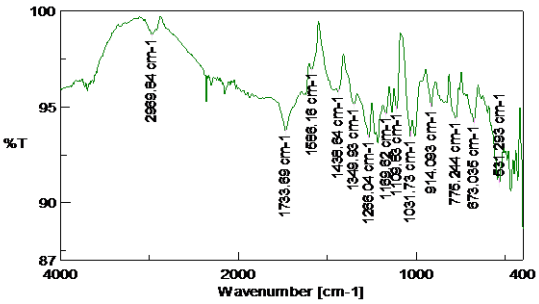

Fig. 1: FTIR analysis of *S.molesta*

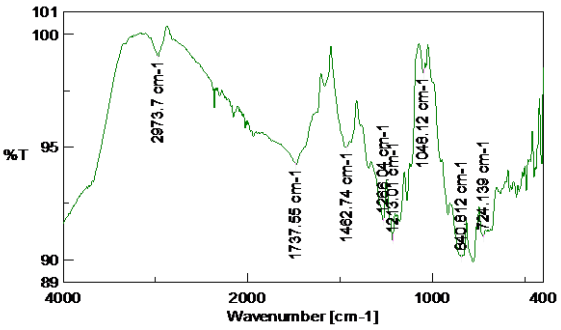

Fig. 2: FTIR analysis of *C.pyrenoidosa*

(ii) ***Thermogravimetric analysis of S. molesta and C. pyrenoidosa:*** According to Sudhakar and Premalatha (2015) the weight loss due to moisture removal from the sample occurs at 190°C followed by the vaporization of organic matter and initiation of thermal degradation of the sample within the range of 28-470°C. Finally, the slow decompostion of the solid residue occurs at 730°C. As shown Fig. 3 and 4, the thermogravi-metric analysis of *S. molesta* and *C. pyrenoidosa* consists of three distinct stages. In the first stage is the drying period in which water were removed for the temperature below 200°C. The second stage called as devolatilization occurs at temperature between 200 to 500°C. During this stage, a remarkable slope of TG curve was observed due to significant drop in weight of samples with liberation of volatile hydrocarbon from rapid thermal decomposition of hemicelluloses, cellulose and some part of lignin. The weight of the sample is reduced to less than 50% which indicates that 80 %weight of the sample contains volatile fraction and the solid carbonaceous residue is around 20 %weight. In the third stage, the weight loss is primarily due to steady decomposition of the remaining heavy components of the sample like lignin.

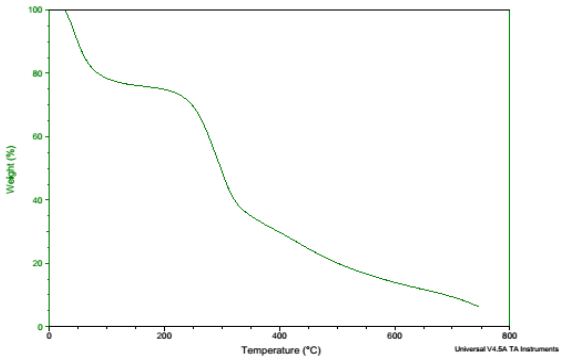

Fig. 3: Thermogravimetric analysis of *S.molesta*

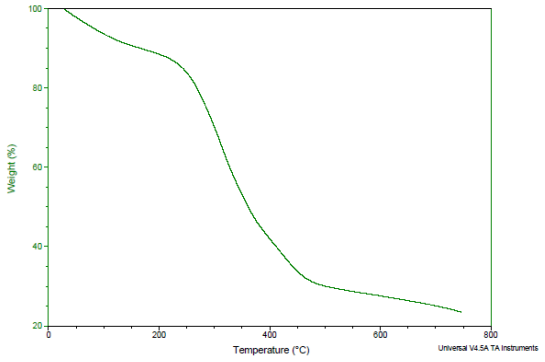

Fig. 4: Thermogravimetric analysis of *C.pyrenoidosa*

Table 2: Measurement of mass loss percentage as a function of temperature

Temperature °C	*S.molesta* Mass loss (%)	*C. pyrenoidosa* Mass loss (%)
29-100	21.52	6.38
100-200	4.51	5.48
200-300	34.71	20.77
300-350	28.44	24.30
350-400	14.96	20.86
400-500	32.49	28.49
500-550	17.04	4.42
600-700	32.58	9.06
700-750	32.71	6.25

CONCLUSIONS

The characterization of *S. molesta* and *C. pyrenoidosa* was done using FTIR and thermogravimteric analysis. Using FTIR spectra, the relative lipid content and carbohydrate content of *S. molesta* and *C. pyrenoidosa* were 1.498, 1.797 respectively was identified. Also, the functional groups of ester and fatty acids were identified in *S. molesta* and *C. pyrenoidosa* which explores the possibilities of using these two as biodiesel feedstock. The presence of functional groups of carbohydrate, cellulose is predominant which indicates that *S. molesta* and *C. pyrenoidosa* can be used bioethanol production. The thermogravimetric analysis showed the presence of three distinct stages such as drying, devolatilization and steady decomposition of heavy components such as lignin (Imen Abed et al. 2012).

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REFERENCES

Andrew P. Dean, David C. Sigee, Beatriz Estrada, Jon K. Pittman, Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. Bioresour Technol. **101**(12): 4499-4507 (2010).

Bi, Z. and B.B. He, Characterization of microalgae for the purpose of biofuel production. Transactions of the ASABE **56(4):** 1529-1539 (2013)

Dean, A.P., David C. Sigee, Beatriz Estrada and J. K. Pittman, Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. Bioresource Technology **101:** 4499–4507 (2010)

Dilek (Yalcin) Duygu, Abel U. Udoh, Tulay (Baykal) Ozer, Aydin Akbulut, Ilkay (Acikgoz) Erkaya, Kazim Yildiz and Deniz Guler, Fourier transform infrared (FTIR) spectroscopy for identification of Chlorella vulgaris Beijerinck 1890 and Scenedesmus obliquus (Turpin) Kützing 1833. African Journal of Biotechnology **11**(16): (2012).

Ferreora, A.F., A.P. Soares Dias, C.M. Silva and M. Costa, Evaluation of thermochemical properties of raw and extracted microalgae. Energy **92**: 365-372 (2015).

Figueira, C.E., Paulo Firmino Moreira Jr., Reinaldo Giudici, Thermogravimetric analysis of the gasification of microalgae Chlorella vulgaris. Bioresour Technol **198**: 717-724 (2015).

Gai, C., Zhengang Liu, Guanghua Han, Nana Peng and Aonan Fan, Combustion behavior and kinetics of low-lipid microalgae via thermogravimetric analysis. Bioresour Technol **181**: 148-154 (2015)

Imen Abed, M.P., Khaled Loubar, Fethi Zagrouba, Mohand Tazerout, Thermogravimetric investigation and thermal conversion kinetic of typical North African and middle eastern lignocellulosic waste. Bioresources 7(1): 1200 -1220 (2012).

Liu, J., Yanfei Pan,Changhong Ya, HaitaoWang, Xupeng Cao, SongXue. Determination of ash

content and concomitant acquisition of cell compositions in microalgae via thermogravimetric (TG) analysis. Algal Research **12**: 149-155 (2015).

Laurens, L.M.L. and E.J. Wolfrum, Feasibility of Spectroscopic Characterization of Algal Lipids: Chemometric Correlation of NIR and FTIR Spectra with Exogenous Lipids in Algal Biomass. BioEnergy Research **4**(1): 22-35 (2010).

Meng Y., Changhong Yao, Song Xue and Haibo Yang, Application of Fourier trans-form infrared (FT-IR) spectroscopy in determination of microalgal compositions. Bioresour Technol **151**: 347-354 (2014).

Mubarak, M., A. Shaija and T. V. Suchithra Optimization of lipid extraction from Salvinia molesta for biodiesel production using RSM and its FAME analysis. Environmental Science and Pollution Research **23**: 14047-14055 (2016).

Mubarak, M., A. Shaija and T.V. Suchithra, Cost Effective Approach for Production of Chlorella pyrenoidosa: A RSM Based Study. Waste and Biomass Valorization (2018) https:// doi.org /10.100 7/s12649-018-0330-x.

Mubarak, M., A. Shaija., T.V. Suchithra, Ultrasonication: An effective pre-treatment method for extracting lipid from Salvinia molesta for biodiesel production. Resource-Efficient Technologies **2**: 126-132 (2016).

Mubarak, M., A. Shaija and T.V Suchithra, A review on the extraction of lipid from microalgae for biodiesel production. Algal Research **7**: 117-123 (2015).

Sudhakar, K. and M. Premalatha, Characterization of Micro Algal Biomass Through FTIR/TGA /CHN Analysis: Application to Scenedes-mu ssp. Energy-Sources, Part A: Recovery, Utilization and Environmental Effects **37**(21): 2330-2337 (2015).

Tang, YT., Xiao Qian Ma and ZhiYi Lai, Thermogravimetric analysis of the combustion of microalgae and microalgae blended with waste in N2/O2 and CO2/O2 atmospheres. Bioresour Technol **102**(2): 1879-1885 (2011).

Zabaniotoua, A., O. Ioannidoua, E. Antonakoub and A. Lappasb, Experimental study of pyrolysis for potential energy, hydrogen and carbon material production from lignocellulosic biomass. International Journal of Hydrogen Energy **33**(10): 2433-2444 (2008).