Hydrothermal Liquefaction of Microalgae (Spirulina Platensis) under Subcritical Water Conditions for Bio-fuel Production

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Abstract— In this study bio- crude oil with H/C ratio resembling that of petroleum crude oil was produced via hydrothermal liquefaction of wet Spirulina platensis under subcritical water conditions. Factorial design and response surface methodology were used in synthesis of bio-crude oil. The experimental design was performed to study the effect of the variables (time &temperature) on the process and interaction among variables, while the response was the % yield by weight of produced bio-fuel. It was found that the model agreed well with the experimental data. The higher heating value of the bio -crude oil was estimated to be about 35.77MJ kg-1.The elemental composition of the liquefied Spirulina platensis was comparable to that of petroleum crude oil with the exception of the oxygen and nitrogen content being higher in the bio-oil. Thus upgrading of the liquefied micro algae especially deoxygenating and denitrogenating is necessary.

Index Terms— bio crude oil, factorial design, subcritical water temperature, Spirulina platensis, thermo-chemical liquefaction.

I. INTRODUCTION

Biomass can be converted by several processes to intermediate solid, liquid or gaseous bio-fuels that are said to have the potential supplying 30% of the global fuel demand without affecting the food production [1]

Many pathways currently under consideration for producing liquid fuels from biomass to supplement the fossil-based liquid transportation fuels [2]. The most promising among these are the algae-based advanced bio-fuels. The unique advantages of using algae feedstock for producing advanced bio-fuels include: (a) high productivity of lipids (b) minimizes competition with conventional agriculture (c) utilizes a wide variety of water sources (d) recycles stationary emissions of carbon dioxide (e)compatible with integrated production of fuels and co products within bio-refineries[3]-[5].

Biomass processing by thermo chemical methods includes liquefaction, gasification and pyrolysis. Gasification is a process of partial oxidation at high temperatures to produce gaseous fuel products. Pyrolysis involves the production of bio-oil, gases and charcoal in the absence of air at medium to high temperatures. Hydrothermal liquefaction (HTL) is a process in which biomass is converted in hot compressed water to a liquid bio- crude. Processing temperature range from 200 to 350ºC with pressure around 15-20 MPa, at these conditions water has remarkable properties as a reaction medium to assist the complex biomass molecules to break down to smaller molecules that may re-polymerize to oily compounds[6]. HTL process is promising process for converting wet biomass into crude bio-oils or bio-crude with or without catalyst [7],[8].

The products of hydrothermal liquefaction of biomass consist of a bio-crude fraction, a water fraction containing some organic and inorganic compounds, a gas fraction and a solid residue fraction. The bio-crudes product from microalgae usually have a high heating value and can be further upgraded to other conventional liquid transportation fuels including jet fuels [9]-[13].

The advantage of hydrothermal liquefaction is that it can process both dry and wet biomass, so biomass feedstock drying is no longer needed. Since microalgae biomass usually has high moisture content, the drying process requires a large amount of thermal energy due to the high heat of vaporization of water [14]. The liquefaction studies were performed at subcritical temperatures (below 374ºC) and gasification of algae biomass often in supercritical water [15].

The mechanism of hydrothermal liquefaction has not yet been elucidated. The influence of various factors like temperature, holding time or ash content remains still unclear, but in hot compressed liquid water close to the critical point (below 374ºC and 22.1MPa), competition between two reactions takes place hydrolysis and polymerization [16]. The first one has more importance at the early stages of the process when the microalgae are decomposed and de-polymerized to small compounds. These compounds may be highly reactive, thus polymerization and forming bio- crude, gas and solid compounds [17],[18]. When increasing the reaction time or temperature, re-polymerization, condensation and decomposition of the compounds from different phases may occur, this may led to an increase of the solid and gas yields and reduction of the bio-crude oil yield[19].This is consistent with the fact that bio-crude viscosity decreases with increasing holding time [20].

The conventional approach for the optimization of a multivariable system is usually one-variable-at-a-time. However, such approach needs to carry out numerous sequential experimental runs and cannot explain the interactions between the variables. Recently many statistical experimental design methods have been employed in bioprocess optimization. Response surface methodology (RSM) is one of such scientific approach [21]-[24]. It is an effective statistical technique dealing

with the development and non-parametric simulative models. This model approximates the functional relationships between input variables and the output (response) of the process using experimental data [25]. It is a powerful tool that involves the following general advantages; more information per experiment than unplanned approaches, a reduction in the number and cost of experiments, it makes possible the calculation of the interactions among experimental variables within the range studied, leading to a better knowledge of the process and it facilitates the determination of the operating conditions necessary for the scale-up of the process [26].

The objective of the present work was to evaluate the different variables that affecting the hydrothermal liquefaction of microalgae (*Spirulina Platensis*) under subcritical water conditions for bio-fuel production using factorial design and response surface methodology, and to determine the physical and chemical properties of the product.

II. EXPERIMENTAL

A. Raw feedstock

Local strains of microalgae Spirulina platens (Sp.p.) was obtained from the Algal Biotechnology Unit, National Research Center. Acetone ultra-pure 99% was obtained from Sigma Cosmetic.

B. Preparation of biomass for biochemical analysis

The ash content was determined by ashing the algae using pre-ignition followed by oxidation in the muffle furnace at 575^0 C [27].The analysis of Spirulina platensis was tested to determine the elemental composition of the organic fraction based on C, H, N and S with O calculated by difference. The effective hydrogen to carbon ratios for any biomass feedstock and fuel is calculated [28] by the following equation:

$$\frac{H}{C} = \frac{N(H) - 3N(O)}{N(C)}$$

Where N (H), N (O) and N(C) are the stochiometric number of H, O and C They were estimated by dividing the compositional value of a given atom with its corresponding atomic weight. Since more negative value suggests that the sample has a small heating value. The effective H to C ratio is an indication of the potential of this biomass as a feedstock for fuel production.

From Dulong formula [29], the higher heating value (HHV) was calculated as follows:
HHV (MJ kg^{-1}) = 0.3383C+ 1.422(H - O/8) +0.095S

Where C, H and O are the mass percentages of carbon, hydrogen and oxygen respectively

C. Liquefaction apparatus

The liquefaction process was performed in a reactor made of stainless steel 316 vessel (400 ml capacity) provided with a pressure gauge and surrounded with external electric heater. All the autoclave is equipped with a control panel.

D. Experimental procedure

In each experimental run 50 gm of dry algae sample was mixed with 200 ml of distilled water and homogenized for 5 minutes at 1000 rpm then charged into the reactor. The heating rate was approximately 10°C min-1. The reaction time was started when the mixture reached required temperature. After each run, the reaction vessel was suddenly cooled in iced water to room temperature. The reaction mixture was filtered, and then the bio-oil in the solid phase was extracted using acetone followed by filtration after washing with additional 100 ml of acetone and shaking for about 30 minutes. The solids were dried and weighed. Four products from algae were obtained: gases, organic soluble water phase, solvent soluble phase (bio – crude) and solid residue, as shown in figure (1). The oil phase was evaporated using a rotary evaporator, yielding a dark – brown viscous material referred as bio – crude.
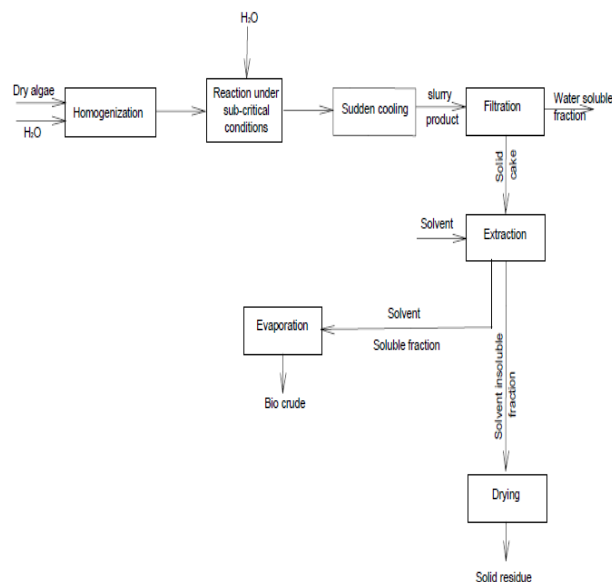

Fig (1) :Block Flowsheet for algae hydrothermal liquefaction

E. Analysis of algae, algal oil

E.1. Gas Chromatography Mass (GC/MS)

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadruple MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness).For GC/MS detection, an electron ionization system with ionization energy of 70 eV (electron volt) was used. Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set

at 280 ^{0}C. The oven temperature was programmed at an initial temperature 40 ^{0}C (hold 3 min) to 280 ^{0}C as a final temperature at an increasing rate of 5 ^{0}C /min

E.2. Elemental analysis

An Element analyzer (Elemental Viro- El, Germany) was used to analyze the bio crudes and solid residue samples. All samples are dried at - 105°C till constant weight.

E.3 Calorific value

The bio – crude samples were dried at 105°C for 24 hrs before the test, then the calorific value was measured in the Egyptian Petroleum Research institute using a calorific value tester (Parr 6200 ,2010) and also calculated using Dulong formula as mentioned before[29].

F. Estimation of bio-crude fuel oil yield

The yield was obtained using the percentage of converted microalgae and subtracting the fractions corresponding to the acetone insoluble without considering ash content and assuming that the subtracted value (gases+ solids+ aqueous soluble organics) has possible effect as fuel as shown in the following equation [30],[31]:

$$\%F = \frac{B}{C-(G+S+A)} \times 100$$

Where: F= fuel oil mass fraction, B= weight of bio-crude, C=weight of feed algae, G=gases, S=solids, A=aqueous soluble organics

G. Statistical analysis

G.1. Basic principle of RSM

In recent years, response surface design is an effective data processing method used in the design of experiments to deal with multi variable effect based on the mathematical statistics theory. The greatest merit is that it can be used to determine the nonlinear relationship between the dependent and independent variables, and to analyze the interactive effects of independent variables on dependent variables. A complete Response surface methodology (RSM) model [21]-[24], including constant, linear, interacted, squared terms can be expressed as:

$$Y = a_0 + \sum_{k=1}^{2} a_k X_k + \sum_{k=1}^{2} a_{kk} X^2_k + \sum_{k=1}^{2} a_{kj} X_k X_j$$

Where a_0 is the intercept, a_k is the first-order model coefficient, a_{kk} is the quadratic coefficient for the ith variable, and a_{kj} is the interaction of variables k and j. The independent variables chosen were time and temperature while the response was the % yield (Y) of produced bio-fuel. The variables selected and their levels were shown in table1

Table (1) Variables selected and their levels for response surface design

Variables	Coded factor levels				
	-1.414(-α)	-1	0	+1	+1.414(+α)
Reaction temperature	201	225	283	340	364
Reaction time	24	30	45	60	66

III. RESULTS AND DISCUSSION

A. Statistical analysis

In order to optimize the reaction factors a five- level two factors central composite rotatable design was utilized in this study. To gain information regarding the interior of the experimental region and to evaluate the curvature, this study was conducted in a total of 12 experiments in accordance with a 22 complete fractional design plus four central point's coded as '0' (for experimental error estimation) and four axial points coded ±α (star points). Where α is the distance from the origin to the star point it is given by α=2n/4 (in this design n = 2 and α=1.414).

B. Optimization of reaction conditions

Experimental data for five level-two factor response surface analysis is shown in table 2.We conducted the 12 designed experiments and analyzed the results with multiple regressions using design expert 6 software. All the runs were performed at random.

The response function for the main effects and interaction was fitted to a 2F1mode [21]-[25] and the following equation was obtained:

yield% =43.5+2.06* temp+3.26* time-10.99* temp *time(1)

Table (2) Experimental data for five level-two factor response surfuce analysis

Standered	Run	Factor1 Temp. ^{0}C	Factor2 Time,min	Response Yield %
2	1	+1	-1	60.86
4	2	+1	+1	41.73
6	3	0	0	44
3	4	-1	+1	56.9
1	5	-1	-1	32.06
5	6	0	0	44
12	7	0	0	44
11	8	0	0	44
7	9	-α	0	37
8	10	+α	0	39

10	11	0	+ α	46.4
9	12	0	-α	32

To check the adequacy of the empirical model, a statistical analysis of the model was performed. The results of ANOVA [21]-[24] for the selected factorial model are summarized in table3. ANOVA also represents the interaction of variables on the response as well as the effect of each individual variable

Table(3) ANOVA for Response Surface 2FI Model, Analysis of Variance Table [Partial sum of squares]

Source	Sum of squares	Degree of freedom	Mean square	F value	p-value Prob> F	
Model	602.19	3	200.73	13.79	0.0025	Significant
Temperature, A	*33.86*	1	33.86	2.33	0.1711	
Time ,B	*84.99*	1	84.99	5.84	0.0463	
Temp. *Time ,AB	*483.34*	1	483.34	33.20	0.0007	
Residual	101.90	7	14.56			
Lack of Fit	101.90	5	20.38			
Pure Error	0.000	2	0.000			
Total	819.1	11				

The response function for the main effects and interaction was fitted to a 2F1model [21]-[25] and the following equation was obtained:
yield% =43.5+2.06* temp+3.26* time-10.99* temp *time(1)

The P-values are used as a tool to check the significance of the corresponding coefficient. The smaller the P values are, the bigger the significance of the corresponding coefficient [32].The Model F-value of 13.79 implies the model is significant. There is only a 0.25% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The "Adequate Precision" statistic is computed by dividing the difference between the maximum predicted response and the minimum predicted response by the average standard deviation of all predicted responses. Large values of this quantity are desirable, and values that exceed four usually indicate that the model will give reasonable performance in prediction [33]. In this study; the ratio was 11.574 (from ANOVA), which means that the model can be used to navigate the design space.

The statistical model obtained from Eq. (1) can be represented as three-dimensional surface and contour plots as shown in Figures (2-3). These show the response surface and predicted values of the yield as a function of temperature and time. In addition, the coefficient determination (R^2=0.86) and adjust coefficient of determination (Adj. R^2=0.8) indicated that, the accuracy and general availability of the model were adequate.
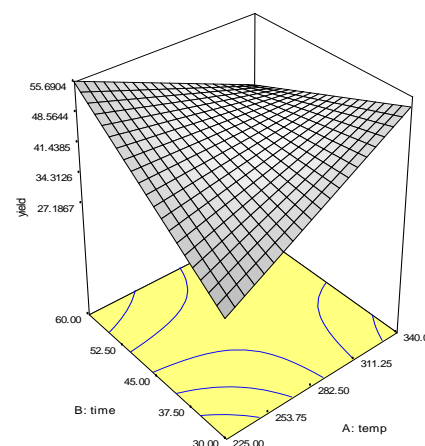
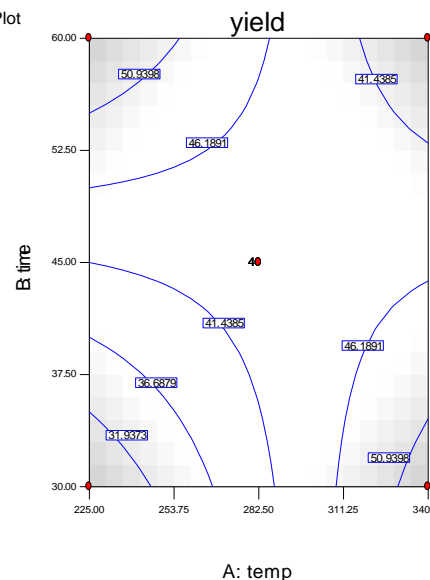**Fig (2)Response surfuce of yield versus temperature and time**

Fig (3)Combined effect of temperature and time on yield

Figure (4) shows a plot of the residual distribution which defined as the difference between calculated through the model and observed values versus the observed values for the studied response: % yield. The quality of fit is good as the residual distribution does not follow a uniform trend with respect to the predicted variables. All the residuals are less than 3%, which indicates that the models adequately represent the yield over the range of study.
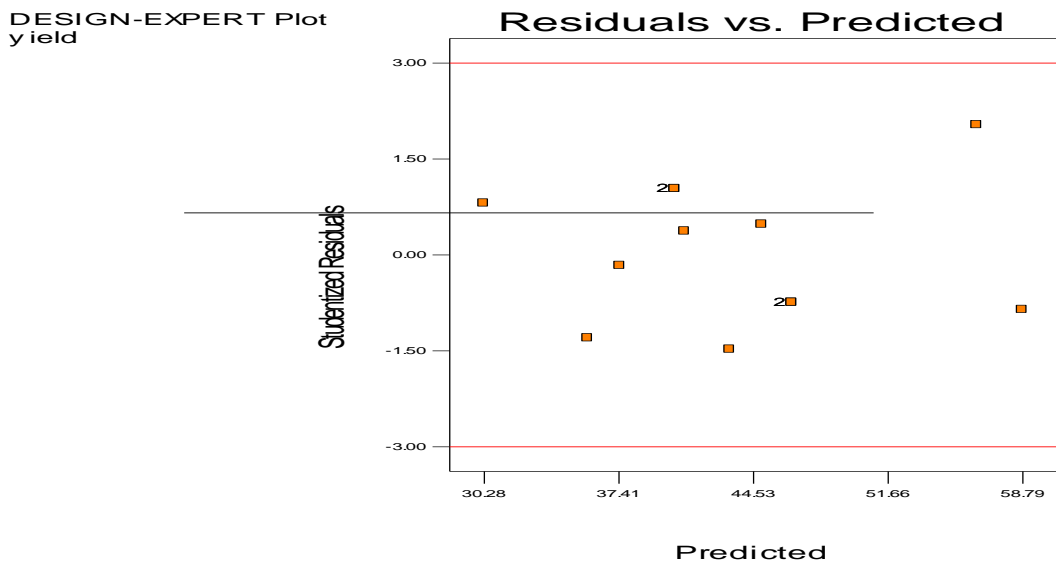

Fig (4) Residual vs. predicted yield

Figure (5) shows the predicted and experimental value using the model equation, which demonstrates that the model was successful in capturing the relation between the parameters to the response and confirmed by the coefficient determination (R^2) value 0.86.
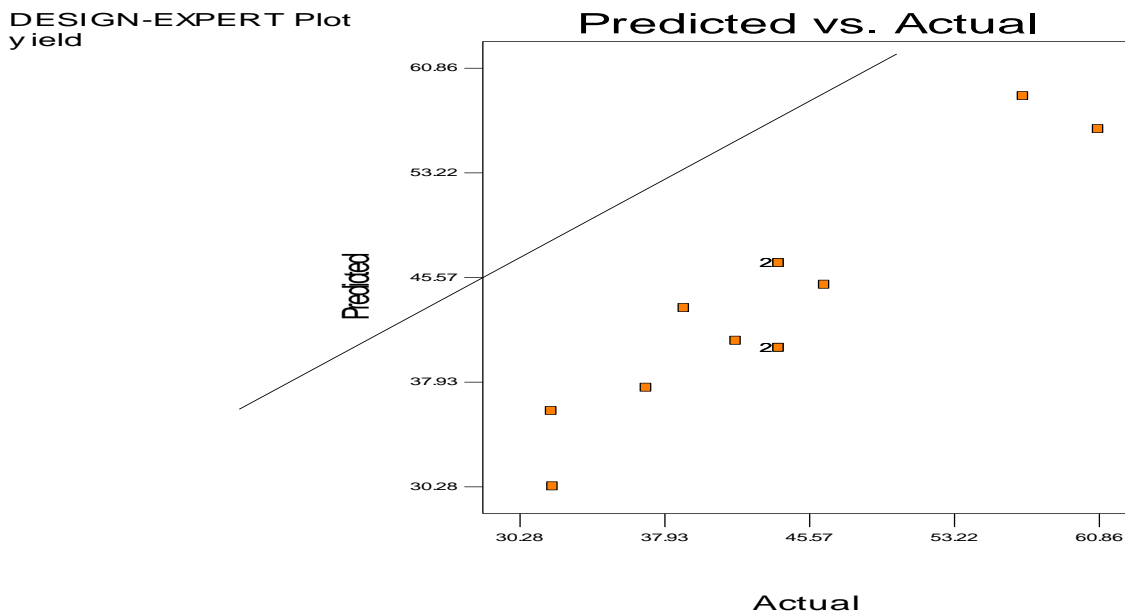

Fig (5) Predicted model versus actual (measured response)

C. Hydrothermal liquefaction conversion

From the elemental composition values listed in table (4) the effective hydrogen to carbon ratio for micro algae (*Spirulina platensis*) is estimated to be (-0.016). It is expected from the estimated effective hydrogen to carbon ratio for *Spirulina platensis* is suitable feedstock for liquid transportation fuel production. Table (5) illustrates the elemental composition values of *Spirulina platensis* after hydrothermal liquefaction.

The elemental analysis results clearly show that hydrothermal liquefactions produces a bio-oil that enriched in carbon and hydrogen and has reduced levels

of nitrogen and oxygen compared to the original algae biomass feedstock. This elemental composition is comparable to that of petroleum crude oil with the exception of the oxygen and nitrogen content being higher in the bio- oil. Thus upgrading of the liquefied micro algae especially deoxygenating and denitrogenating is necessary. Another way to interpret the elemental composition data is in terms of atomic ratios (e.g., H/C and O/C). Ratio of H/C to the dry algal biomass is 2. While the bio-oil produced at subcritical temperatures have less values. These values compare favorably to H/C ratios in petroleum crude oil [34]. The O/C ratio in the bio-oil decreased steadily with the liquefaction temperature from 0.69 for the original algal to 0.074.

Table (4): Elemental composition (wt %) of two micro algae

Algae Name	Oil content %	Ash%	C%	H%	O%	N%	S%	Effective H/C*	HHV MJ/Kg
Spirulinapl antensis	9	15	40.54	7.01	37.695	8.879	6.076	-0.016	17

*Effective H/C is calculated as mentioned before (28)

Table (5) Elemental composition (wt %) of bio- oil via temperature and time

Run	Temp. ^{0}C	Time min	C	H	O	N	S	H/C	O/C	N/C
Sp.p			40.54	7.01	37.69	8.879	6.54	2.075	0.697	0.188
3	283	45	66.57	7.21	18.009	7.3	0.022	1.300	0.203	0.094
6	283	45	69.63	8.85	14.107	7.46	0.053	1.525	0.152	0.092
7	283	45	71.9	9.51	11.61	7.4	0.029	1.587	0.121	0.088
10	364	45	73.15	9.13	11.089	6.44	0.041	1.498	0.114	0.075
2	340	60	62.02	6.76	27.62	5.63	0.012	1.308	0.334	0.078
1	340	30	70.71	9.6	17.13	6.69	0.059	1.629	0.182	0.081
11	283	66	66.24	8.4	20.75	6.62	0.12	1.522	0.235	0.086
8	283	45	52.27	6.85	33.76	8.34	0.069	1.573	0.484	0.137
4	225	60	58.26	7.26	25.43	8.71	0.031	1.495	0.074	0.128
12	283	24	66.18	9.02	16.89	7.804	0.014	1.636	0.191	0.101

Therefore liquefaction performs a significant content of deoxygenation but additional deoxygenation is required to produce a fungible transportation fuel. The bio-oil yield increased with increasing temperature till 340°C then it decreased again and this is agreed with Diego et al (30). The % yield is increased than the oil content of the algae due to the extra conversion of protein and carbohydrate components within the cell of microalgae.

D. Effect of reaction time and temperature on solid residue yield

It can be seen from Table (6) that the reaction time had a more pronounced effect on the solid residue yield at low temperature than at high temperature. The decrease of the solid residue with reaction time implied that the solid matter of *Spirulina platensis* was active and subjected to decompose in HTL and further decomposition could be achieved with increasing the reaction time. Therefore, extending the reaction time can increase the organic conversion and thus the oil yield but with low quality.

So, we can produce the same amount of oil from *Spirulina platensis* at high temperature with a short reaction time or at low temperature with a long reaction time. These results agree with He et al. and Dote et al [35], [36].

Table (6) Effect of reaction time and temperature on solid residue

Run	Temperature (C°)	Time (min.)	Solid (Wt %)
4	224	60	8.7
11	283	66	7.7
12	283	24	20.4

6	283	45	10.1
1	341	30	13.1
8	362	45	9.3

E. Higher heating value (HHV)

The results of bio- oil yields and higher heating value (HHV) at each temperature presented in table (7). Despite the varying of bio-crude oil yields, and bulk elemental analysis (table 5) resulted in only small difference in estimated higher heating values of the product oils ranging from (25.40 to 35.88 (MJ kg^{-1})).These values are slightly lower than that of petroleum crude oil (43 MJ kg^{-1}) but significantly higher than that of dry microalgae feedstock (17 MJ kg^{-1}).

Table (7) Bio crude oil yield and higher heating value

Run	Bio-Oil Yields (Wt %)	HHV (MJ kg^{-1})
4	56.9	25.48
12	32	32.2
11	46.4	30.7
3	44	33.65
6	44	29.58
7	44	35.88
1	60.86	34.55
2	41.73	25.69
10	39	35.77

F. Physico–chemical characterization

The most prevalent peaks in the spectrum were primary and secondary amines as tetradecamide (C14), Hexadecamide (C16), and Dodecamide (C11), (C12). This may result from the decomposition of proteins found in carbon chain backbone with amine. Also, Spirulina plantensis produce bio-crude oil that contains phenolic and ketonic groups. The major compounds are cyclo alkene and saturated hydrocarbon chain alkane. The phenol compounds are likely derived from the carbohydrate and protein fraction which have been identified during HTL.Similarly the fatty acid and cholesterol compounds are likely derived during the decomposition of triglyceride under (HTL) conditions. The high hetero atom content of HTL compounds is a major concern particularly in the low boiling fraction.

IV. CONCLUSIONS

In this study bio-crude oil was produced by the hydrothermal liquefaction of microalgae (Spirulina Platensis) under subcritical water conditions implementing response surface methodology method to optimize the reaction conditions.

Effect of temperature and time on the bio-fuel oil yield was investigated. 2F1mode fits the data for the bio-fuel oil yield. The study of the factors affecting the response show that, within the experimental range considered, the most important factor is the time. Analysis of the residuals and analysis of the observed values versus the predicted ones demonstrated the efficiency of the model obtained.

The results demonstrate that high lipid content of algae not the exclusive choice for future to bio-fuel applications.

HTL conversion of low lipid content algae into bio-crude oil could be quite promising for achieving effective algae bio-fuel.

Based on our experimental data the highest bio – oil yield (60% wt) was achieved at reaction temperature of 340°C with reaction time 30 minutes via hydrothermal liquefaction of Spirulina platensis.

The high heteroatom content of bio –crude oils will likely lead to upgrading requirements before use.

Recommended as a future enhancement, new experiments with different micro algae are needed to develop the influence of cell structure and composition on the process yield.

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