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Biocrude oil production and nutrient recovery from algae by two-step hydrothermal liquefaction using a semi-continuous reactor

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Abstract−We evaluated two-step hydrothermal liquefaction in a semi-continuous reactor for recovery of both nutrients and biocrude from the alga *Coelastrum* sp. in direct comparison with a one-step process. The influence of the operating temperature, pressure and water flow rate was investigated by means of a 2^k factorial experimental design and response surface methodology. The two-step process gave a higher total biocrude yield (~36 wt% (daf. basis)) and nutrient recovery level in terms of nitrogen containing compounds (~60 wt% of the protein content in the original algae as ammonium and nitrate ions and protein/polypeptides) than the single-step process. The highest biocrude yield was achieved at first-step temperature of 473 K, second-step temperature of 593 K, pressure of 200 bar and water flow rate of 0.5 mL/min.

Keywords: Hydrothermal Liquefaction, Algae, Nutrient Recovery, Biocrude, Response Surface Methodology

INTRODUCTION

The increasing global energy consumption coupled with the declining non-renewable fossil fuel reserves and concern over the ecological and environmental carbon footprint of the use of these fossil fuels is driving an increased interest in and requirement for the increased production of alternative fuels to coal, petroleum and natural gas. The production processes for liquid biofuels, including pyrolysis oil (biooil and, hereafter, biocrude), bioethanol and biodiesel, have been explored over the past few decades [1-6]. The two important thermochemical processes for liquid biofuel production are liquefaction and pyrolysis that convert the biomass into biofuel within a short residence time and in a single step.

Hydrothermal liquefaction is a relatively environmentally friendly technique for converting biomass into biofuels under hot compressed water or subcritical water [7,8]. Hot compressed water or subcritical water is water at a moderate to high temperature (373-648 K) that maintains its liquid state under high pressure. Its properties, including the solubility, density and dielectric constant, can be adjusted by changing the temperature and pressure and allow it to be a highly reactive medium [9]. There are several advantages of hydrothermal liquefaction compared to other thermal processes. It does not require a pretreatment process such as drying, for the raw material and other additives [8,10-12]. Moreover, the produced biocrude can be self-separated from the water when the reaction returns to the standard condition, atmospheric pressure and room temperature. The hydrothermal liquefaction of algae has been investigated since the algae have high photosynthetic activity and growth rate compared to other biomass, leading to an increased CO_2 absorption. It can grow in a wide variety of water resources and have a high moisture content that is suitable for use in this process [9, 11,13-16]. The suitable conditions for biocrude production have been reported to include a reaction temperature of ~553-623 K, giving a 15-50 wt% yield of biocrude depending upon the alga species and its composition.

Since algae have a high protein content, there is the possibility of recovering some of the nitrogen-based nutrient content from the algae [17]. Subcritical water has been reported to be suitable media for protein and amino acid extraction from biomass [18-21]. However, the suitable temperature for protein extraction has been reported to be less than 523 K, which is lower than that required for hydrothermal liquefaction. Investigation of the nutrient recovery from algae along with/without biocrude production using a hydrothermal process has been reported [12,13,22]. For example, two-thirds of the nitrogen and up to 85% of the phosphorous content in the algae could be recovered in the aqueous phase [22]. In addition, the processed water, which had a high concentration of nutrients, including ammonium and nitrate ions, could be used as growth media for further algae cultivation [13]. The hydrothermal process can be used as a pretreatment process for nitrogen

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removal prior to biocrude production. The combination of a hydrothermal pretreatment followed by pyrolysis revealed that the biocrude obtained from the pretreated algae had a higher carbon and lower nitrogen content compared to that produced without pretreatment, resulting in a higher heating value of the biocrude [19].

However, most of the studies mentioned above conducted experiments in a batch reactor. We explored the hydrothermal process using a semi-continuous system to avoid thermal decomposition of the product and to stimulate flexibility in the process operation. Experiments were performed based on a response surface methodology and 2^k factorial design. The process was operated in two steps with different operating temperatures and pressure. The first step operated at low temperatures, 423-493 K, was considered as a pretreatment step for nutrient recovery. The second step was introduced as a subsequent process operated at relatively high temperature (553 and 593 K) for biocrude production. A 2^k factorial experimental design and response surface methodology (RSM) were used to examine the influence of the operating temperature, pressure and water flow rate. In addition, the product distribution obtained from the two-step process was compared to that obtained from the one-step process. The factor screening and selection for different purposes was suggested as well.

MATERIALS AND METHODS

1. Raw Materials and Alga Characterizations

The dried alga, *Coelastrum* sp., was provided by the PTT Research and Technology Institute and kept at room temperature. The ultimate analysis was conducted using a CHN analyzer (CHN-2000, LECO Instrument (Thailand) Ltd.) for the total carbon/hydrogen/nitrogen levels. Oxygen was calculated from the remaining sample mass balance (dry, ash-free basis). The protein content in original algae was estimated by multiplying the total nitrogen content by the conversion factor of 4.44 [23]. Proximate analysis was performed by ASTM E870-82. The results of ultimate and proximate analysis are presented in Table 1.

Table 1. Composition of the original *Coelastrum* sp. alga

Composition	wt%
Proximate analysis (as received)	
Moisture	9.10
Volatile matter	46.75
Fixed carbon	9.41
Ash	34.74
Ultimate analysis (dry, ash-free basis)	
C	23.62
H	4.15
N	3.46
O (by difference)	34.02
Total	100.00
Other components (dry, ash-free basis)	
Protein	15.36

2. Experimental System and Procedures

The two-step hydrothermal liquefaction process was performed and compared to the one-step process in a semi-continuous reactor. A schematic layout of the reactor and procedure is illustrated in Fig. 1. In the reactor (SUS316, OD: 0.5 inch and 0.083 inch of thickness), the alga was packed along with some of inert material (gravel) to prevent the compaction of the raw material after water feeding. Water was introduced into the reactor by an analytical isocratic HPLC pump (PU-2080, JASCO Ltd.). The pressure and temperature were then adjusted with a back-pressure regulator (BP-66, GO regulator) and tube furnace (CTF12/65/550, Carbolite Ltd.), respectively. For the one-step hydrothermal liquefaction process, after the pressure and temperature reached the desired setting values the product that passed through the back-pressure regulator was collected for 2 h. The liquid product was separated using dichloromethane (DCM), where the DCM insoluble fraction was defined as aqueous co-products and the DCM soluble fraction was defined as the biocrude. The solvent in the DCM soluble fraction was recovered by vacuum evaporator at 323 K, and then the weight of the residual biocrude was measured. The nutrients, in terms of the level of ammonium (NH_4^+) and nitrate (NO_3^-) ions and polypeptides, in the aqueous co-products were then analyzed as described in section 2.3.

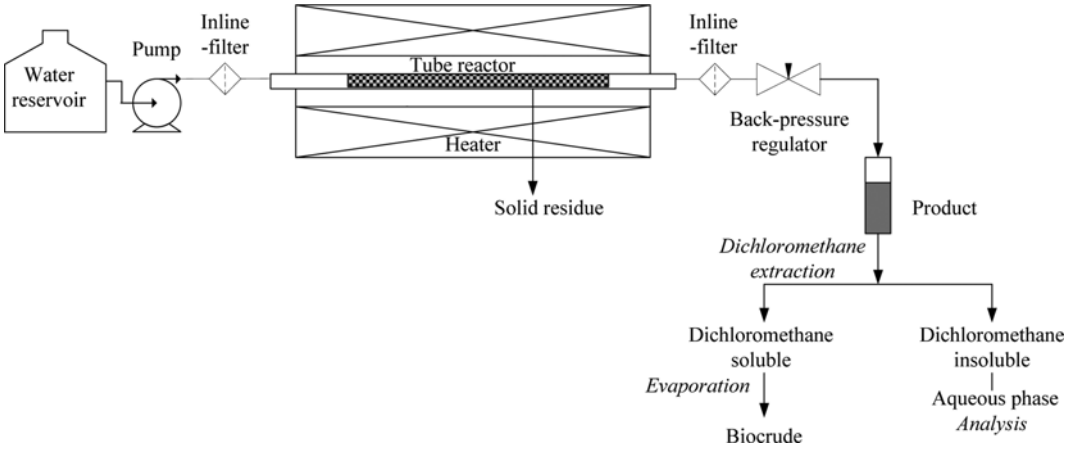

Fig. 1. Schematic layout of the reactor and experimental procedure used.

For the two-step hydrothermal liquefaction process, the operating procedure was similar to the one-step process above, except that after the liquid product of the first low temperature step had been collected for 2 h, the pressure and temperature were then increased to the desired values of the second hotter step and the liquid products of this step were then also collected over 2 h. The pressure of the first step was 70 bar. The liquid products obtained from both steps were separated and analyzed by the same procedure as described above.

3. Analytical Methods and Calculations

After product collection, the %solid residue and %biocrude were calculated by using Eqs. (1) and Eq. (2), respectively:

$$\%\text{Solid residue} = \frac{\text{wt. of solid residue} - \text{wt. of ash}}{\text{wt. of daf. algae}} \times 100 \quad (1)$$

$$\%\text{Biocrude} = \frac{\text{wt. of biocrude oil}}{\text{wt. of daf. algae}} \times 100 \quad (2)$$

where daf. stands for dry-ash free basis. The total polypeptide level was determined by Lowry's method using BSA as a standard [24]. The sample was diluted and mixed well with the analyzing Folin & Ciocalteu's phenol reagent, and the absorbance was then measured at 750 nm with a UV spectrophotometer. The extractable polypeptide yield was reported in terms of the %nitrogen recovery compared to the amount of nitrogen content in original alga, as demonstrated in Eq. (3),

$$\%\text{N}-\text{polypeptide} = \frac{\text{wt. Protein from Lowry assay}}{6.25 \times \text{wt. of N in original algae}} \times 100 \quad (3)$$

The total NH_4^+ level was spectrophotometrically determined following the reaction of ammonia with salicylate and hypochlorite to produce indophenol blue following the method of [25] and measuring the absorbance at 660 nm. Total NO_3^- was determined using the spectrophotometric screening method as reported [26], measuring the absorbance at 220 nm and then dividing this by twice the absorbance reading at 275 nm. The NH_4^+ and NO_3^- yields are reported in terms of the %nitrogen recovery compared to the amount of nitrogen content in the original algae, as presented in Eqs. (4) and (5), respectively:

$$\%\text{N}-NH_4^+ = \frac{\frac{NH_4^+(\text{mg/L})}{mw.NH_4^+} \times \text{mw.N} \times \text{vol. of Aqueous phase}}{\text{wt. of N in original algae}} \times 100 \quad (4)$$

$$\%\text{N}-NO_3^- = \frac{\frac{NO_3^-(\text{mg/L})}{mw.NO_3^-} \times \text{mw.N} \times \text{vol. of Aqueous phase}}{\text{wt. of N in original algae}} \times 100 \quad (5)$$

4. Response Surface Methodology (RSM) Based 2^k Factorial Design

To evaluate the effects of the reaction temperature, pressure and water flow rate on the product distribution, a 2^k factorial design was applied to both the one-step and two-step processes. The one-step process was divided into a low and high temperature phase and so considered two factors and three factors, respectively (Table 2(a)), while the two-step process considered four factors (Table 2(b)). The data were then analyzed by analysis of variance (ANOVA) to identify the important factors and/or their interactions that had potentially significant effects on the product yields, accepting significance at a probability level of $p \leq 0.05$. The regression model was then generated and used to evaluate the RSM using Eq. (6) [27],

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum\sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad (6)$$

where y is the predicted response, β_0 is the constant coefficient, β_i is the linear regression coefficient in the regression model, β_{ij} is the regression coefficient of interaction between two factors, X_i and X_j represent the coded variables and ε represents the error term.

Using ANOVA and regression analyses gives an effective determination of the optimal conditions for the desired products, especially in the case of considering several targets simultaneously. In this work, the desired product for the one-step process conducted at a low temperature was polypeptides that could potentially be recovered at 423-473 K using subcritical water. The desired product for the one-step process conducted at a high temperature was biocrude. Finally, for the two-step process, the nutrients were the optimal products in the first step while biocrude production was the desired second step product. The optimal conditions for each case were proposed after RSM analyses.

Table 2. The factors and their levels for the hydrothermal liquefaction of *Coelastrum* sp.

(a) For one-step process at a low and high temperature

Factor	Coded symbol	Level (uncoded symbol)	
		−1	1
Low temperature			
Temperature (K); T_1	X_1	423 (a)	473 (A)
Water flow rate (mL min^{-1}); F_1	X_2	0.5 (b)	1.0 (B)
High temperature			
Temperature (K); T_2	X_1	553 (a)	593 (A)
Pressure (bar); P	X_2	120 (b)	200 (B)
Water flow rate (mL min^{-1}); F_2	X_3	0.5 (c)	1.0 (C)

(b) For two-step process

Factor	Coded symbol	Level (uncoded symbol)	
		−1	1
Temperature (K); T_1	X_1	423 (a)	473 (A)
Temperature (K); T_2	X_2	553 (b)	593 (B)
Pressure (bar); P	X_3	120 (c)	200 (C)
Water flow rate (mL min^{-1}); F	X_4	0.5 (d)	1.0 (D)

RESULTS AND DISCUSSION

1. Effect of the Operating Variables on the Nutrient Recovery and Biocrude Production in the One-step Process

The effect of each of the three principal operating factors upon

Table 3. The different products recovered from the one-step hydrothermal liquefaction of *Coelastrum* sp.

(a) At low temperature							
Code	T_1 (K)	F_1 (mL min^{-1})	N-NH_4^+ (%)	N-NO_3^- (%)	N-PP (%)	Total N[a] (%)	Solid residue (%)
ab	423	0.5	1.1	0.4	5.0	6.5	51.4
Ab	473	0.5	2.4	1.0	14.0	17.4	38.1
aB	423	1.0	1.4	0.6	7.9	9.9	56.0
AB	473	1.0	3.2	1.5	32.6	37.3	40.1
(b) At high temperature							
Code	T_2 (K)	P (bar)	F_2 (mL min^{-1})	Biocrude (%)	N-NH_4^+ (%)	N-NO_3^- (%)	N-PP (%)
abc	553	120	0.5	20.7	10.6	1.6	20.4
Abc	593	120	0.5	22.1	12.4	1.6	14.9
aBc	553	200	0.5	27.2	8.4	1.5	15.9
ABc	593	200	0.5	29.5	12.5	1.5	13.8
abC	553	120	1.0	23.6	9.6	2.0	24.8
AbC	593	120	1.0	21.1	11	1.7	21.0
aBC	553	200	1.0	15.0	9.8	2.0	24.0
ABC	593	200	1.0	16.5	13.1	1.7	24.9

[a]Total soluble nitrogen (recovered level), derived as the sum of the %N-NH_4^+, %N-NO_3^- and %N-polypeptide levels
Data are the average from two replicates. PP=protein/polypeptide

the nutrient recovery and biocrude production level was evaluated in the one-step hydrothermal liquefaction process. The nutrient recovery was carried out at a low temperature (423 and 473 K), while the biocrude production was carried out at a higher temperature (553 and 593 K). The effect of each factor and the deduced important operating factors were discussed in sections 3.2.1 and 3.2.2.

1-1. Nutrient Recovery at Low Temperature

Table 3(a) shows the % nitrogen recovery in the aqueous co-products in terms of the level of ammonium (%N-NH_4^+) and nitrate (%N-NO_3^-) ions and polypeptide (%N-PP) levels, as well as the %total nitrogen recovery of the %solid residue. The results indicated that increasing the temperature from 423 K to 473 K increased the NH_4^+ and NO_3^- levels about two-fold while the polypeptide level was increased about three- to four-fold. On the other hand, the solid residue was decreased. This likely reflects the increased level of hydrolysis of protein in the original algal feedstock [28]. Moreover, the rate of thermal degradation of protein into soluble polypeptides and further degraded products, such as amino acids, NH_4^+ and NO_3^-, was accelerated. The nitrogen recovery was also found to increase with an increased water flow rate from 0.5 to 1.0 mL min^{-1} at both temperatures. This was probably due to the increased water/algae ratio that results in an increased mass transfer of products into the aqueous co-products.

1-2. Biocrude Production at a High Temperature

The %biocrude yield and %N recovery in the form of nutrients and %solid residue levels obtained at the high temperature (553 and 593 K) are summarized in Table 3(b). With respect to the nutrient recovery, a different trend was observed compared to that obtained at the lower temperature. The polypeptide yield (%N-PP) was decreased while the ammonium ion yield (%N-NH_4^+) was increased as the temperature was increased from 543 to 593 K.

Considering the net %N-NH_4^+ plus %N-PP level obtained (37.3%) at 473 K and a 1 mL min^{-1} water flow rate (Section 3.2.1), this was almost equal to those levels obtained in all conditions at high temperatures (Table 3(b)), which ranged between 25.8-39.7%. Thus, most of the extractable polypeptide was recovered at temperatures above 473 K. However, a higher NH_4^+ yield and a lower polypep-

Table 4. ANOVA test for the responses

(a) For %N recovery as polypeptides from the single-step process					
Source	Sum of squares	DF	Mean square	F-value	*P*-value>F
Model	923.39	3	307.80	104.80	0.0003
X_1	569.07	1	569.07	193.77	0.0002[a]
X_2	230.49	1	230.49	78.48	0.0009[a]
X_1X_2	123.83	1	123.83	42.16	0.0029[a]
Residual	11.75	4	2.94		
Total	935.13	7			
(b) For the %biocrude production level at a high temperature in the single-step process					
Source	Sum of squares	DF	Mean square	F-value	*P*-value>F
Model	331.24	6	55.21	14.55	0.0004
X_1	1.81	1	1.81	0.48	0.5070
X_2	0.12	1	0.12	0.03	0.8656
X_3	136.06	1	136.06	35.87	0.0002[a]
X_1X_2	5.89	1	5.89	1.55	0.2440
X_1X_3	5.79	1	5.79	1.53	0.2480
X_2X_3	181.57	1	181.57	47.86	<0.0001[a]
Residual	34.14	9	3.79		
Total	365.38	15			

[a]Significant F-values at the 95% confidence level (p-value≤0.05). DF=Degrees of freedom

tide yield were obtained at the higher temperature, suggesting that thermal degradation of the extractable polypeptide by polypeptide hydrolysis and amino acid deamination took place at 553 and 593 K.

With respect to biocrude production, no significant effect of the temperature was clearly observed (Tables 3(b) & 4(b)). This indicates that the effect of temperature was quite small compared to the other operating factors at this temperature range. Moreover, an interaction between the pressure and water flow rate was observed. At a lower pressure (120 bar), increasing the water flow rate from 0.5 to 1.0 mL min^{-1} did not significantly change the biocrude yield. However, at a higher pressure (200 bar) the biocrude yield was dramatically decreased with an increased water flow rate. These phenomena can be explained by the competition between the two main secondary reactions of thermal cracking and repolymerization that occurred along with the cage formation under the hot compressed water atmosphere. The cage effect is the formation of water around the intermediate products that then hinders their further decomposition but favors their repolymerization instead [28].

At a lower pressure, the water density was not high enough to form a cage properly, and so secondary reactions, including repolymerization and thermal cracking, occurred simultaneously. The change in the biocrude yield was not dominated by either repolymerization or thermal cracking alone, even when the water flow rate was changed, resulting in a comparable biocrude yield.

On the other hand, at a higher pressure or water density, the water could form a cage around the intermediate product. Repolymerization then occurred, leading to an increased biocrude yield (~1.3-fold at both 553 and 593 K). However, when the water flow rate was increased, the residence time was decreased and became insufficient to allow the water to form a cage. Repolymerization was then hindered, while the higher water density promoted the degradation reactions, such as hydrolysis, leading to a decreased biocrude yield (~1.8-fold at both 553 and 593 K).

1-3. RSM and ANOVA Results

At low temperature, ANOVA analysis demonstrated that the temperature (X_1), water flow rate (X_2) and their interaction all had a significant effect on the %N-PP recovered (Table 4(a)). The %N recovery of polypeptides was selected as the representative readout because it had the highest value among all nutrients. The derived coded regression model to estimate the predicted value of the polypeptide level is shown in Eq. (7),

$$y=14.87+8.43X_1+5.37X_2+3.93X_1X_2 \quad (7)$$

The coefficient of variation (R^2) was almost unity (0.99), supporting a very good agreement between the predicted (using Eq. (7)) and experimental values. The coefficients of all terms indicated the positive effect of these factors on the level of %N-PP recovery. The generated three dimensional (3-D) surface and contour plots showed the effect of temperature and water flow rate and their in-

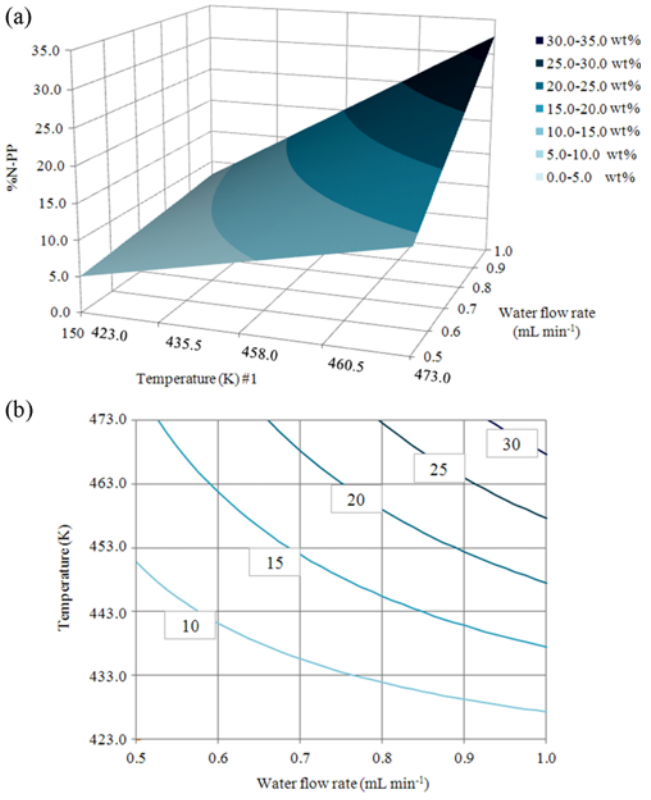

Fig. 2. (a) Three-dimensional response surface and (b) contour plot of the effects of the first stage temperature (#1) and water flow rate on the %N recovery as polypeptides in the single-step hydrothermal liquefaction at a low temperature.

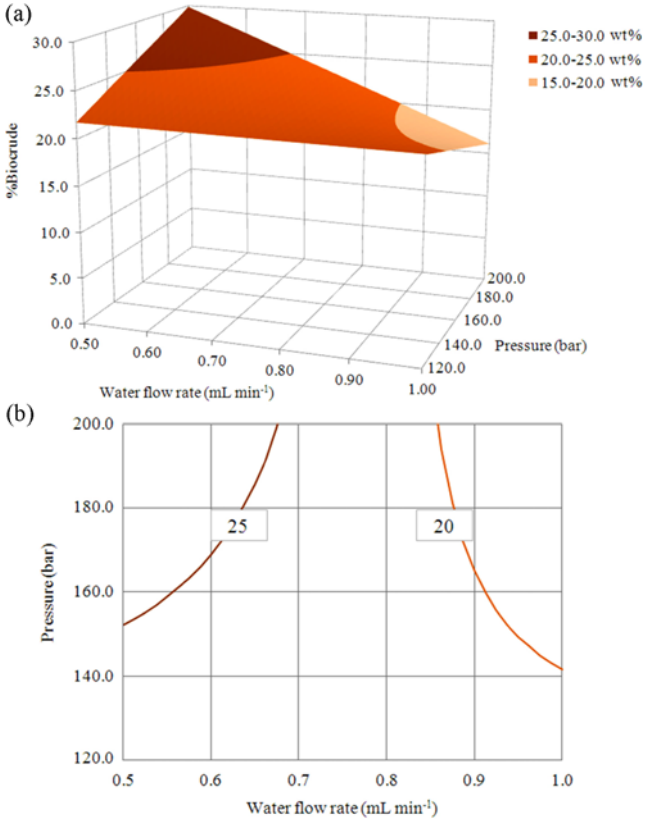

Fig. 3. (a) Three-dimensional response surface and (b) contour plot of the effects of the pressure and water flow rate on the %biocrude yield at 593 K in the single-step hydrothermal liquefaction.

teraction on the level of polypeptides (%N-PP) (Fig. 2). These results clarified the interaction effect of the reaction temperature and water flow rate. A higher polypeptide level was achieved at a higher temperature and water flow rate.

At high temperature (Table 4(b)), the derived coded regression model for the biocrude yield, shown in (Eq. (8), had an R^2 value of ~0.91 indicating a good agreement with the experimental values. The resulting 3D-response surface and contour plots (Fig. 3), which illustrated the effect of the pressure and water flow rate on the obtained biocrude yield, revealed the clear interaction between these two operating factors. A higher biocrude yield (>25%) was achieved at a higher pressure while keeping the water flow rate lower.

$$y=21.95+0.34X_1+0.085X_2-2.92X_3 +0.61X_1X_2-0.60X_1X_3-3.37X_2X_3 \quad (8)$$

2. Effect of Operating Variables on the Nutrient Recovery and Biocrude Production Levels in the Two-step Process

The two-step hydrothermal liquefaction process was explored in order to extract the nitrogen containing compounds or nutrients in the first step and produce biocrude in the second step. Table 5 summarizes the operating conditions and product yields. The nitrogen-containing compounds were recovered in the first step, and the remaining solid was then extracted to produce biocrude in the second step. The biocrude level obtained in the second step was about 15-24 wt%, which was lower than that obtained in the one-step process (15-30 wt%). This was because some of the biocrude (9-18 wt%) was produced during the first step, and so the total recovered biocrude from the two-step process (27-36 wt%) was higher than that obtained from one-step process (15-30 wt%). Presumably the light hydrocarbons could be extracted in the first step and the heavy hydrocarbons were then generated at the higher temperature of the second step. The two-step operation would thus prevent or reduce the decomposition of the light biocrude fractions at higher temperatures and so result in an increased total biocrude yield. Considering the %nutrient recovery, not all of the nitrogen containing compounds were recovered from the algae in the first step with some remaining in the solid residue that was recovered in the second step. Therefore, the two-step hydrothermal liquefaction process gave a higher yield of both biocrude and nitrogen containing compounds compared to the one-step process.

The obtained general coded regression model for the proposed two-step process is shown in Eq. (9):

$$y_i=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_4+\beta_{12}X_1X_2+\beta_{13}X_1X_3 +\beta_{14}X_1X_4+\beta_{23}X_2X_3+\beta_{24}X_2X_4+\beta_{34}X_3X_4+\varepsilon \quad (9)$$

where y_i is the predicted responses for the product yields reported in Table 5, and β_i and β_{ij} represent the estimated coefficients of each factor and their interaction (Table 6). These coefficients were used to evaluate the effect of the first step temperature and the second step temperature, pressure and water flow rate and their interactions. The high values of R^2 suggest that the model fitted well with the experimental values.

The effect of each term, including the relative magnitude and direction, in terms of the estimated coefficients is shown in Table 6, where the relatively high estimated coefficients represent a high influence of that factor compared to the others. The estimated coefficients for the effect of temperature (X_1) on the %biocrude, %N-NH_4^+ and %N-PP levels in the first step were all high and positive,

Table 5. The products recovered from the two-step hydrothermal liquefaction

Code	T_1 (K)	T_2 (K)	P (bar)	F (mL min^{-1})	Biocrude #1 (%)	Biocrude #2 (%)	Total biocrude (%)	N-NH_4^+ #1 (%)	N-NH_4^+ #2 (%)	N-PP #1 (%)	N-PP #2 (%)	Protein conversion[a] (%)	Solid residue (%)	Conversion into aqueous phase[b] (%)
abcd	423	553	120	0.5	10.9	21.1	32.1	1.0	5.8	7.4	15.5	35.6	32.9	29.7
Abcd	473	553	120	0.5	13.2	18.8	32.0	2.0	3.9	17.8	11.7	45.0	20.7	35.4
aBcd	423	593	120	0.5	11.3	22.2	33.4	1.0	8.9	7.0	19.8	42.2	19.2	36.6
ABcd	473	593	120	0.5	13.0	18.0	31.0	1.8	6.2	13.3	11.6	38.8	19.9	32.8
abCd	423	553	200	0.5	9.2	19.4	28.6	1.1	7.0	8.3	12.1	32.3	20.5	28.5
AbCd	473	553	200	0.5	14.3	20.1	34.4	2.2	5.1	14.6	12.4	41.6	15.4	34.1
aBCd	423	593	200	0.5	9.9	23.6	33.5	1.2	9.6	5.0	14.9	32.5	16.2	30.8
ABCd	473	593	200	0.5	13.6	22.7	36.4	2.4	7.1	14.5	10.7	39.7	13.6	34.6
abcD	423	553	120	1.0	11.1	19.8	30.9	1.8	5.6	10.0	19.6	45.5	18.4	36.9
AbcD	473	553	120	1.0	12.1	15.6	27.7	3.0	4.8	26.6	2.0	44.2	16.0	36.4
aBcD	423	593	120	1.0	9.9	19.8	29.6	1.6	9.5	11.1	17.2	44.9	14.6	39.5
ABcD	473	593	120	1.0	10.3	17.3	27.6	2.8	7.0	25.0	12.3	57.5	18.0	47.1
abCD	423	553	200	1.0	12.4	16.3	28.7	1.6	6.5	8.8	16.2	39.2	20.1	33.2
AbCD	473	553	200	1.0	15.8	16.2	31.9	3.2	5.3	24.0	11.9	55.1	16.7	44.4
aBCD	423	593	200	1.0	9.6	18.6	28.3	1.6	7.4	9.0	12.1	33.6	16.7	30.1
ABCD	473	593	200	1.0	17.6	17.3	34.9	3.3	7.3	16.4	11.4	43.9	19.4	38.4

[a]Calculated from the (wt. of ammonium ion+wt of polypeptide)/wt of original protein×100

[b]Calculated from 100% - %solid residue - %total biocrude

Data are the average from two replicates. PP=Protein /polypeptide

#1 and #2 are the first and second step respectively

Table 6. Estimated coefficients for the regression models representing the predicted values of product yields

	Estimated coefficients									
	Biocrude #1	Biocrude #2	Total biocrude	N-NH_4^+ #1	N-NH_4^+ #2	N-PP #1	N-PP #2	Protein conversion[a]	Solid residue	Conversion into aqueous phase[b]
β_0	12.10	19.17	31.28	1.97	6.68	13.66	13.21	41.98	18.64	50.09
β_1	1.62	−0.92	0.70	0.60	−0.86	5.34	−2.72	3.77	−1.18	0.48
β_2	−0.20	0.76	0.56	−0.01	1.18	−1.00	0.53	−0.34	−1.45	0.89
β_3	0.64	0.10	0.74	0.10	0.23	−1.09	−0.50	−2.23	−1.32	0.58
β_4	0.17	−1.57	−1.40	0.39	0.00	2.69	−0.37	3.51	−1.15	2.55
β_{12}	0.11	−0.18	−0.07	0.00	−0.12	−0.70	0.46	−0.40	1.71	−1.64
β_{13}	0.95	0.72	1.68	0.07	0.13	−0.55	1.60	1.59	0.13	−1.81
β_{14}	0.03	−0.09	−0.07	0.10	0.28	1.28	−0.71	0.95	1.22	−1.15
β_{23}	0.15	0.53	0.68	0.07	−0.25	−0.34	−0.97	−1.97	0.60	−1.29
β_{24}	−0.23	−0.12	−0.34	−0.02	−0.06	0.02	−0.12	−0.18	1.13	−0.79
β_{34}	0.81	−0.61	0.20	−0.05	−0.27	−0.71	0.57	−0.31	2.05	−2.25
R^2	0.73	0.69	0.63	1.00	0.96	0.96	0.68	0.74	0.81	0.71

[a]Calculated from the (wt. of ammonium ion+wt of polypeptide)/wt of original protein×100
[b]Calculated from 100% - %solid residue - %total biocrude
Data are the average from two replicates. PP=Protein/polypeptide
#1 and #2 are the first and second step, respectively

suggesting that increasing the temperature in the first step had the most significant effect on increasing these responses. On the other hand, the estimated coefficients of temperature in the first step revealed a negative effect on the %biocrude, %N-NH_4^+ and %N-PP levels obtained in the second step, indicating that a higher temperature in the first step gave a lower yield in the second step. This was because the higher temperature gave higher levels of biocrude, NH_4^+ and NO_+^- in the first step and so lowered these yields in the second step. The operating temperature of second step had a positive effect on the obtained %biocrude, %N-NH_4^+ and %N-PP levels in the second step, but the directions and magnitude of some effects in the two-step process were different from those in the one-step process. This probably reflects that some of the compounds were recovered in the first step, and so the different composition of the residual solid in the second step.

Since the effect of the operating parameters on both the nutrient recovery in the first step and the biocrude production in the second step must be considered, a combined contour plot between the yield of %N-PP in the first step and %biocrude in the second step was generated (Fig. 4). The interaction between the temperature of the first and second step was revealed, but although the optimum condition could not be determined exactly from this graphical plot, it exhibited the trend of each yield at different operating conditions. To obtain a higher biocrude yield from the second step, the temperature of the first step should be kept low while the temperature of the second step should be high. However, the opera-

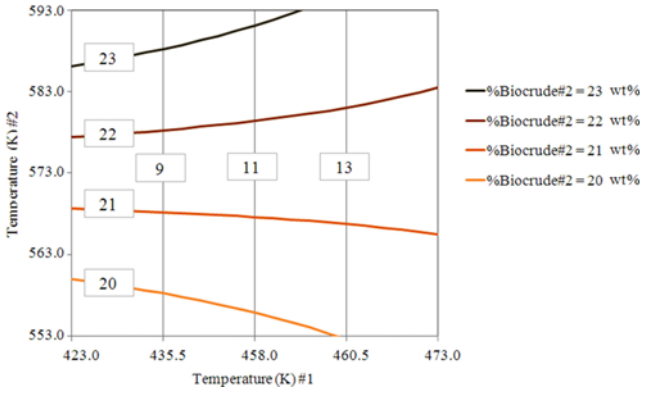

Fig. 4. Superimposed contour plot showing the effect of the first (#1) and second (#2) stage operating temperature on the %N recovery as polypeptide (vertical lines) and the %biocrude yield in a two-step hydrothermal liquefaction. Pressure= 200 bar, water flow rate=0.5 mL min^{-1}.

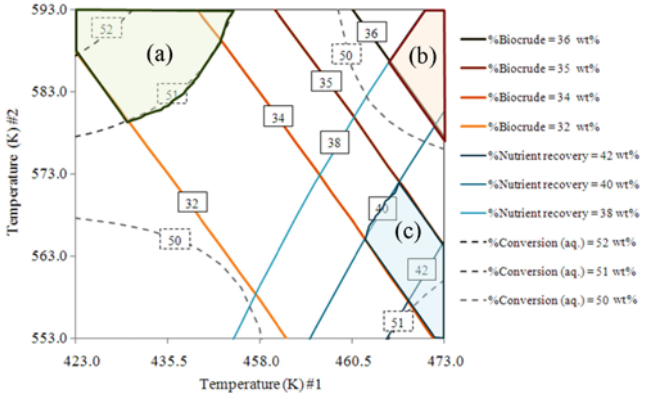

Fig. 5. Superimposed contour plot showing the effect of the first (#1) and second (#2) stage operating temperatures on the %total biocrude, %nutrient recovery from the original protein and %conversion into aqueous phase in the two-step hydrothermal liquefaction. The shaded overlapping area for the three different cases ((a), (b) and (c)) is marked. Pressure= 200 bar, water flow rate=0.5 mL min^{-1}.

tion of both steps at a higher temperature provided a high nutrient recovery in the first step and a moderate biocrude yield in the second step.

3. Optimization of the Conditions for Nutrient Recovery and Biocrude Production in the Two-step Process

The influence of the operating factors on the total biocrude yield and nutrient recovery levels from both steps of the two-step process was also considered. A contour plot of the %total biocrude, %nutrient recovery from original protein and %conversion into aqueous co-products (Fig. 5) was generated from the coded regression model (Eq. (9)), with the estimated coefficients given in Table 6. The resulting contour plot is shown in Fig. 5. The linear lines with labels of 32, 34, 35 and 36 represent the total biocrude yield of about 32, 34, 35 and 36 wt%, respectively. The linear lines with labels of 38, 40 and 42 represent %nutrient recovery from original protein of about 38, 40 and 42 wt%, respectively. The dashed line represents %conversion into aqueous co-products.

This contour plot shows the trend of the product distribution at different operating conditions, where the results are separated into three cases. In case A, the first liquefaction step was performed at 423-450 K whilst the second step was conducted at about 580-593 K. The algae were converted into aqueous co-products more than 51 wt% with total biocrude yield of about 32-34 wt%. However, the nutrients, in the form of protein and NH_4^+, were relatively low compared to the other two cases (B and C; see below). The aqueous co-products obtained from case A might principally contain other nitrogen compounds, such as amino acids and other degraded products.

Case B represents when the system was operated at higher temperatures for both steps (over 465 K for the first step and 575 K for the second step) and gave higher total biocrude yield (>36 wt%) and nutrient recovery in the form of protein and NH_4^+ (38-40.5 wt%) compared to case A. On the other hand, aqueous co-products obtained from case B were lower than case A. It indicated that, at 200 bar and 0.5 mL/min, the algae were selectively converted to the biocrude and nutrients. Finally, case C represents a higher temperature in the first step (>460 K) and a lower temperature in the second step (<570 K), and gave a total biocrude yield approximately 34-35 wt% which lay between the yield obtained from case A and B. However, it gave higher level of nutrient recovery. In this case, the nutrients were potentially recovered in the first step and the remaining solid particle was converted to biocrude at the second step. These results provide the basis for screening and selecting the operating condition to optimize the desired product distribution.

CONCLUSION

In the single-step hydrothermal liquefaction process, the nutrients could be recovered at 423 K and 473 K whilst biocrude could be produced at 553 K and 593 K and gave the highest yield of ~30 wt%. The two-step process, resulted in nutrients and biocrude being recovered from both steps with higher total yield compared to those obtained from the one-step process. The proposed regression models had high regression coefficients, indicating a good agreement with experimental results. The resulting contour plot revealed that the operating conditions could be adjusted to recover suitable products for specified purposes. The operation of the first step at high temperature and the second step at low temperature is preferable to promoting the nutrient recovery. On the other hand, high temperature of both steps contributes to higher biocrude yield. Therefore, the regression models based on 2^k factorial design and the contour plots generated for several cases is useful for selecting the suitable operating conditions to reach a desired product.

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