Medium-low temperature hydrothermal hydrolysis kinetic characteristics of concentrated wet microalgae biomass

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Abstract: To improve microalgae biomass utilization efficiency during biofuel production process, medium-low temperature hydrothermal hydrolysis pretreatment was adopted in this study. The pretreatment kinetic characteristics of concentrated wet microalgae *Chlorella vulgaris* biomass (50 g/L) under medium-low temperature hydrolysis (100°C-200°C) were experimentally investigated. The hydrothermal hydrolysis kinetics describing the coupled effects of temperature, initial pressure and retention time then were proposed using response surface methodology (RSM). The maximum carbohydrate yield reached 327.3 mg/g dried biomass under initial pressure of 4 MPa at reaction temperature of 150°C for 120 min. The maximum protein yield (321.5 mg/g dried biomass) was obtained under initial pressure of 4 MPa at reaction temperature of 200°C for 60 min. Based on the hydrothermal hydrolysis kinetic models, it was confirmed that temperature was the most important factor affecting both carbohydrate and protein release during hydrothermal hydrolysis process. Hydrothermal initial pressure and retention time were significant to carbohydrate release, but not to protein release. While, lipid was mainly distributed in microalgae residual and almost did not exist in supernatant (about 8.03 mg/g). And with assistance of mixed hexane and methanol (the ratio of hexane to methanol was 7:3), 67.69% of microalgae lipid was extracted out from hydrothermal hydrolysed microalgae residual (123.3 mg/g dried biomass).

Keywords: hydrothermal hydrolysis, kinetic characteristics, microalgae, medium-low temperature, biofuel, response surface methodology

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1 Introduction

Fossil energy shortage and global warming are two primary global concerns. Green technology can effectively solve both energy and environmental issues[1,2].

Microalgae has been regarded as one of the most promising feedstocks for green biofuel production because its high photosynthetic rate compared to terrestrial plants[3,4]. Biomass can be converted to gaseous and liquid fuels by fermentation in normal temperature and pressure (30°C-35°C, atmosphere)[5,6].

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However, because of the complex microalgae cell wall structure[7], microalgae biomass is hardly digested by bacteria directly to produce biogas through fermentation. To enhance fermentation of microalgae, microalgae cell wall should be broken so that the substance inside cell would be released. Thereafter macromolecular substances (such as cellulose, starch, and protein) hydrolysed into small molecule substances which could be utilized efficiently by bacteria in fermentation process to produce methane and hydrogen[8]. Hence, microalgae biomass pretreatment is necessary before fermentation[7]. Medium-low temperature (100°C-200°C) hydrothermal hydrolysis pretreatment is one efficient pretreatment for its low energy input with heat recovery and needless of organic solvent[9,10]. Under medium-low temperature hydrothermal hydrolysis pretreatment, microalgae cell wall would be broken and the substance inside the cell would release[8].

Fabiana Passos et al.[11] conducted the medium-low temperature hydrothermal hydrolysis experiments using wet microalgae grown in urban wastewater. They found that hydrothermal hydrolysis at 130°C for 15 min could increase organic matter solubilisation by 8%-13%. Meanwhile, fermentation velocity and yield could be increased by 30%-90%, 17%-39% relative to none pretreatment microalgae biomass, respectively. Compared with microwave and ultrasonic pretreatment, hydrothermal hydrolysis pre-treatment of biomass showed a higher increase (60%) in biogas production[12]. They also studied low temperature hydrothermal hydrolysis pretreatment, and found it was beneficial for fermentation with positive net energy[13]. González-Fernández et al.[14] indicated that biogas production from the treated *Scenedesmus* biomass fermentation was increased by 1.6 times compared to untreated biomass. Lara Mendez et al.[15] indicated that methane productivity of fermentation from the hydrothermal hydrolysis treated *Chlorella vulgaris* biomass at 160°C was improved by 64%. Sebastian Schwede et al.[16] verified that medium temperature hydrothermal hydrolysis pretreatment with 22 kW·h thermal energy input could increase by 210 kW·h electric energy and 162 kW·h thermal energy output, and energy input for pretreatment was negligible compared to the benefit. Hence medium-low temperature hydrothermal hydrolysis pretreatment could be an effective method to increase biogas production of microalgae biomass in subsequent fermentation. However, most researches focused on the effect of hydrothermal hydrolysis pretreatment on biogas productivity by fermentation[17,18]. There are few researches focusing on substances of biomass (such as cellulose, starch, and protein) release process. Therefore, it is important to study the substances release process and kinetic characteristics to learn about hydrothermal hydrolysis mechanism.

Hydrothermal hydrolysis pretreatment was always conducted in a closed reactor. There is a unique pressure in closed reactor that matches temperature, named saturation pressure. During the process of heating, liquid water would be gasified to increase the pressure to match the increasing temperature[19], resulting in an increase of microalgae biomass concentration in liquid water. As mentioned above, temperature would affect pressure, while pressure affecting heating process in turn. Moreover, both of them are key factors of hydrothermal hydrolysis pretreatment. And most researches of hydrothermal hydrolysis pretreatment were focused on the effect of temperature on fermentation but seldom on the pressure[17,18]. Therefore, co-effect of temperature and pressure should be investigated.

Response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables, and it can optimize conditions to obtain maximum or minimum response[20]. It is widely used in statistical experimental design of biological and chemical processes. Ho et al.[21] examined and optimized the application of pressurized low polarity water extraction of lignans, protein and carbohydrate from defatted flaxseed meal by RSM. Appiah-Nkansah et al.[22] applied RSM to optimize diffusion conditions of converting sweet sorghum into sugar. Mussatto et al.[23] applied RSM to optimize recover the hemicellulos sugars of spent coffee grounds. Moreover, RSM is efficient for studying co-effect of variables. Therefore, in this study, the coupled effects of temperature, initial pressure and retention time on

hydrolysis process were studied, and the kinetic formulas describing the relationship between carbohydrate/protein production and three factors were proposed by using response surface methodology (RSM). The condition of hydrothermal hydrolysis pretreatment was also optimized to obtain the maximum carbohydrate and protein production.

2 Materials and methods

2.1 Materials

Chlorella vulgaris FACHB-31 was cultivated with optimized BG-11 medium aerated with 5% CO_2[24] and then concentrated by centrifugation at 8000 r/min for 10 min. The concentrated wet microalgae biomass was stored in a freezer at –20°C. The microalgae biomass macromolecular composition was fairly stable, containing 336.6 mg carbohydrate, 423.6 mg protein and 182.3 mg lipid (per gram dried biomass).

2.2 Methods

Carbohydrate content was determined by phenol-sulphuric method[25] and protein was measured by lowry method[26]. To obtain soluble fractions, the samples were centrifuged at 8000 r/min for 10 min (GL-21M, Cence Co., Ltd, China). Lipid in residue and water was extracted using organic solvent with hexane: methanol =7:3 (hexane: 97.0%, LookChem, China; methanol: 99.5%, Chongqing Chuandong Chemical, China)[27,28]. The suspension (containing residue and water) loading was 5%. The mixture was oscillated for 4 h at room temperature, then separated into the organic-solvent and residues layers by 8000 r/min centrifugation for 10 min. Finally, the hexane layer was removed to recover lipid in an air oven. The lipid-extraction yield based on biomass dry weight was measured by reference to the weight of the recovered lipid.

2.3 Hydrothermal hydrolysis pretreatment

Hydrothermal hydrolysis pretreatment experiments were performed in a 50 mL batch high pressure reactor (QN-WCGF-50 mL, Taikang, China) equipped with a magnetic stirrer. The reactor consists of a reaction cylinder and a pressure gauge/valve assembly. To obtain 50 g/L microalgae suspension, deionized water was added into concentrated microalgae. For a typical run, 30 mL suspension was placed inside the cylinder. In order to remove air and prevent secondary reactions, the cylinder was sealed and purged with nitrogen gas at a flow rate of 80 mL/min for 3 min. The reactor was heated to setting temperature (100°C-200°C) at a heating rate of 5°C/min and then held for some time (0-120 min). After the reaction, the cylinder was cooled down to room temperature by soaking in the ice bath.

2.4 Experimental designs

RSM with Box–Behnken was employed to determine the optimum conditions for microalgae hydrothermal hydrolysis. The experimental factors were ascertained based on the preliminary experiments results. The levels of the three retained variables (temperature, initial pressure, retention time) are indicated in Table 1. Each experiment was repeated twice. The relationship between the yield and the three selected variables were approximated by the following third order polynomial function:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_{ii}^2 + \sum_{ij} \beta_{ij} X_i X_j + \sum_{iij} \beta_{ijj} X_{ii}^2 X_j + \sum_{ijk} \beta_{ijk} X_i X_j X_k + \sum_{iii} \beta_{iii} X_{iii}^3 \quad (1)$$

where, y is the calculated response function; X_i is the corresponding actual value of variable; β_0 is the estimated regression coefficient of the fitted response at the centre point of design; β_i is the ith linear coefficient; β_{ii} is the ith quadratic coefficient; β_{ij} is the ijth interaction coefficient; β_{iii} is iiith cubic coefficient; β_{ijk} is the ijkth interaction coefficient; β_{iij} is the iijth interaction coefficient.

The liner and quadratic coefficient, ijth and iijth interaction coefficient were significant, other coefficients were ignorable, the third order polynomial function could simplify as Equation (2):

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_{ii}^2 + \sum_{ij} \beta_{ij} X_i X_j + \sum_{ijj} \beta_{ijj} X_i X_{jj}^2 \quad (2)$$

The liner and quadratic coefficient, ijth interaction coefficient were significant, other coefficients were ignorable, the third order polynomial function could be simplified as second-order polynomial:

$$y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_{ii}^2 + \sum_{ij} \beta_{ij} X_i X_j \quad (3)$$

Table 1 Hydrothermal experimental domain of the Box–Behnken design

Factors	Coded symbols	Levels		
		−1	0	1
Temperature/°C	X_1	100	150	200
Initial pressure/MPa	X_2	0	2	4
Retention time/min	X_3	0	60	120

3 Results and discussion

Table 2 shows the carbohydrate, protein and lipid yield in designed Box-Behnken experiments. The maximum carbohydrate yield reached 327.3 mg/g dried biomass at initial pressure of 4 MPa at hydrothermal temperature of 150°C for 120 min. Carbohydrate yield increased from 51.27 mg/g to 327.3 mg/g dried biomass as temperature increasing from 100°C to 150°C, but decreased to 31.65 mg/g when temperature increased to 200°C. That is mainly because that hydrothermal hydrolysis at 100°C-150°C could damage microalgae cell wall and then carbohydrate could release out. But with a further high hydrothermal temperature, the produced carbohydrate could be reacted with protein by Maillard reaction[29], resulting in a decline of carbohydrate yield. At hydrothermal temperature of 150°C, carbohydrate yield increased from 77.29 mg/g to 164.2 mg/g dried biomass with hydrothermal initial pressure varied from 0 to 4 MPa. However, with a hydrothermal temperature of 200°C, carbohydrate yield did not increase with initial pressure increasing from 0 to 4 MPa. Carbohydrate increased from 134.9 mg/g to 327.3 mg/g dried biomass as retention time varied from 0 to 120 min when the temperature was below 150°C, decreased from 320.9 mg/g to 31.70 mg/g dried biomass as retention time varied from 0 to 120 min at hydrothermal temperature of 200°C. The maximum protein yield (321.5 mg/g dried biomass) was at initial pressure of 4 MPa at hydrothermal temperature of 200°C for 60 min. Protein yield increased with higher hydrothermal temperature, it was 85.30 mg/g dried biomass at 100°C and 321.5 mg/g dried biomass at 200°C. It was different from carbohydrate yield because Maillard reaction consumed much less protein than carbohydrate[25]. Initial pressure of 4 MPa increased by 60 mg protein/g dried biomass at 150°C and 200°C compared to no initial pressure. This is attributed to that protein was consumed by Maillard reaction.

Table 2 Carbohydrate, protein and lipid yield in designed Box-Behnken experiments

No.	Variables			Responses		
	Real values (coded values)[a]			Yield (mg·g^{-1} dried biomass)[b]		
	X_1	X_2	X_3	Carbohydrate	Protein	Lipid
1	150(0)	0(−1)	0(−1)	77.29	85.95	4.35
2	200(1)	0(−1)	60(0)	49.69	256.3	7.53
3	150(0)	2(0)	60(0)	239.3	240.2	7.06
4	150(0)	4(1)	0(−1)	164.2	144.3	3.59
5	150(0)	2(0)	60(0)	246.1	243.6	7.34
6	150(0)	4(1)	120(1)	327.3	230.3	8.03
7	200(1)	2(0)	120(1)	31.65	289.6	8.5
8	200(1)	4(1)	60(0)	41.82	321.5	5.68
9	100(−1)	4(1)	60(0)	140.32	95.62	3.28
10	150(0)	2(0)	60(0)	245.4	248.9	6.8
11	100(−1)	2(0)	0(−1)	134.9	85.25	4.07
12	150(0)	2(0)	60(0)	251.0	243.6	6.72
13	100(−1)	0(−1)	60(0)	169.0	91.30	5.3
14	200(1)	2(0)	0(−1)	320.9	281.8	6.72
15	150(0)	2(0)	60(0)	251.8	229.2	7.4
16	100(−1)	2(0)	120(1)	133.0	104.2	6.59
17	150(0)	0(−1)	120(1)	318.7	211.8	7.13

Note: [a]: X_1: Temperature, °C; X_2: Initial pressure, MPa; X_3: Retention time, min; [b]: carbohydrate, protein and lipid yield in supernatant.

3.1 Carbohydrate released kinetic characteristics

The experimental carbohydrate yield results are shown in Table 2. The results of analysis of variance (ANOVA) for carbohydrate yield are given in Table 3: the model F-value of 569.63 with a low probability (p<0.0001) indicted high significance of the model. The linear effects, quadratic effects, interaction effects listed in Table 3 were all significant by t-test at a level of 0.05. Determination coefficient (R^2) was 0.9994, indicating that the model fitted experimental results well. Adeq precision measured the signal to noise ratio, its value was 68.145 which higher than 4, indicating that the signal was adequate in design space. It showed that the retention time was the most important variable then was the temperature and initial pressure.

As shown in Table 3, liner and quadratic coefficient, ijth and iijth interaction coefficient were significant, other coefficients were ignorable. The relationship between the carbohydrate yield and the three selected variables were approximated by Equation (2).

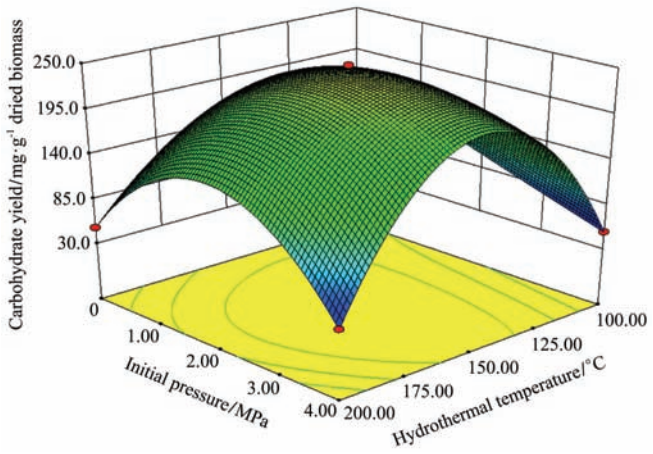
Table 3 ANOVA for carbohydrate yield

Source	Mean square	*F*-value	*p*-value
Model	13123.41	520.44	<0.0001
X_1-Temperature	1792.92	71.10	0.0011
X_2- Initial pressure	2277.72	90.33	0.0007
X_3-Retention time	40901.20	1622.04	<0.0001
X_1X_2	97.90	3.88	0.1201
X_1X_3	20642.53	818.63	<0.0001
X_2X_3	1531.19	60.72	0.0015
X_1^2	47985.00	1902.97	<0.0001
X_2^2	6740.14	267.30	<0.0001
X_3^2	964.63	38.25	0.0035
$X_1^2X_2$	2144.73	85.05	0.0008
$X_1^2X_3$	60484.70	2398.68	<0.0001
$X_1X_2^2$	11361.52	450.57	<0.0001
R^2	0.9994		
Adeq precision	67.319		

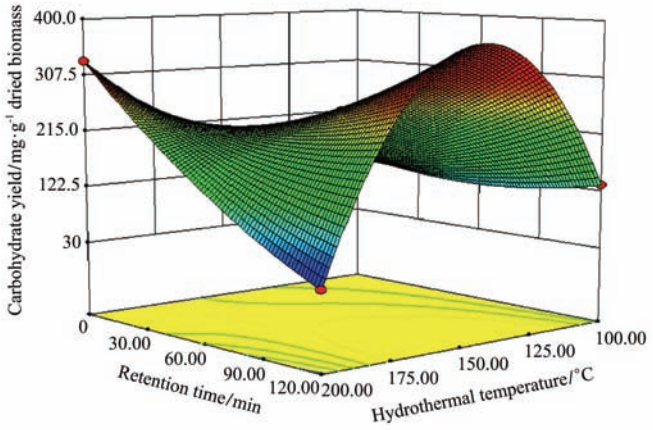
The coefficients in Equation (2) could be calculated by software Design Expert 8.0.6, and the third-order models in term of coded variable result were obtained as Equation (4):

$$Yeild_{carbohydrate} = 246.72 + 21.17X_1 + 23.86X_2 + 101.12X_3 + 4.95X_1X_2 - 71.84X_1X_3 - 19.57X_2X_3 - 106.75X_1^2 - 40.01X_2^2 + 15.14X_3^2 - 32.57X_1^2X_2 - 173.90X_1^2X_3 - 75.37X_1X_2^2 \quad (4)$$

Figure 1 shows that all the variables (temperature, initial pressure and retention time) have important effect on carbohydrate releasing out of microalgae in hydrothermal hydrolysis process. Figure 1a shows the effect of hydrothermal temperature and initial pressure with retention time of 60 min. When initial pressure and retention time were the same, carbohydrate yield would increase when hydrothermal temperature varied from 100°C to 150°C, but decrease when temperature varied from 150°C to 200°C, because Maillard reaction consumed more carbohydrate at higher temperature[29]. Around hydrothermal temperature of 150°C and retention time of 60 min, carbohydrate yield increased with hydrothermal initial pressure varied from 0 to 4 MPa. However, under hydrothermal temperature of 100°C and 200°C, carbohydrate yield did not increase with increasing initial pressure, this because liquid water was pressurized by nitrogen gas. As shown in Figure 1b, when temperature was below 150°C, longer hydrothermal retention time was beneficial to carbohydrate yield. But at 200°C, carbohydrate yield decreased as retention time increased. The explanation is that carbohydrate releasing dominates at low temperature while Maillard reaction takes a leading role at high temperature[29], and longer retention time leads to carbohydrate consumed.



a. Effects of hydrothermal temperature and initial pressure with retention time of 60 min



b. Effects of hydrothermal temperature and retention time with initial pressure of 2 MPa

Figure 1 Surface plots for carbohydrate yield during concentrated wet *Chlorella vulgaris* biomass hydrothermal hydrolysis

3.2 Protein-releasing kinetic characteristics from wet *Chlorella vulgaris* cell by medium-low temperature hydrothermal hydrolysis

The results of ANOVA for protein yield according to experimental protein yields (as shown in Table 2) are given in Table 4. The model *F*-value of 14.90 with a low probability (p=0.0009) revealed that the second-order model fitted the experimental data well. The liner effects, quadratic effects, interaction effects with $p<0.05$ were significant listed in Table 3. Determination coefficient (R^2) was 0.9504, it meant the model fitted experimental results well. Adeq precision value was 12.635 which was higher than 4, indicating that signal was adequate in design space. Temperature had shown

to be the most important variable, then was the retention time, but initial pressure seemed nonessential.

Table 4 ANOVA for protein yield

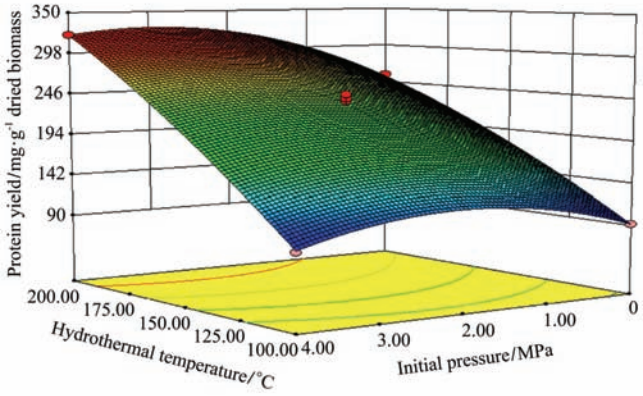
Source	Mean square	*F*-value	*p*-value
Model	10751.13	14.90	0.0009
X_1-Temperature	74662.56	103.50	<0.0001
X_2- Initial pressure	2672.21	3.70	0.0957
X_3-Retention time	7120.18	9.87	0.0163
X_1X_2	924.40	1.28	0.2949
X_1X_3	30.75	0.043	0.8423
X_2X_3	396.88	0.55	0.4824
X_1^2	508.10	0.70	0.4290
X_2^2	4615.22	6.40	0.0393
X_3^2	4892.54	6.78	0.0352
Residual	721.35		
Lack of fit	1434.24	7.68	0.0389
R^2	0.9504		
Adeq precision	12.635		

The relationship between the protein yield and the three selected variables were approximated by the following second-order polynomial function:

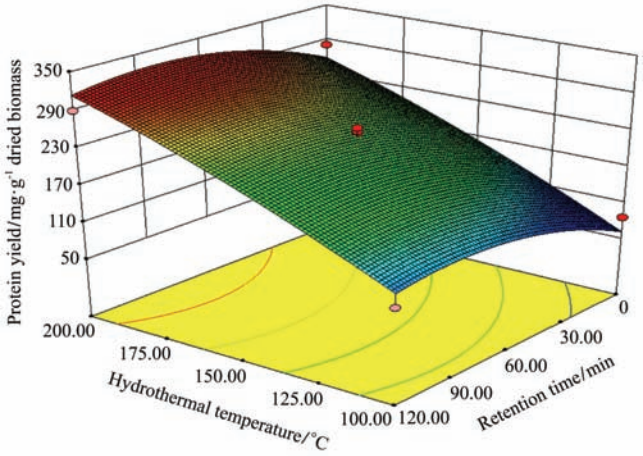
The coefficients in Equation (3) could be calculated by software Design Expert 8.0.6, and the second-order model in term of coded variable result was obtained as Equation (5):

$$\text{Yeild}_{proteins}=235.27+96.61\times X_1+18.28\times X_2+29.83\times X_3+15.20\times X_1\times X_2 -2.77\times X_1\times X_3-9.96\times X_2\times X_3-10.99\times X_1^2-33.11\times X_2^2-34.09\times X_3^2 \quad (5)$$

Figure 2 shows surface plots for protein yield during concentrated wet *Chlorella vulgaris* biomass hydrothermal hydrolysis. As shown in Figures 2a and 2b, protein yield increased with higher hydrothermal temperature. It was different from carbohydrate. Carbohydrate and protein yield performed upsides (approximately 240 mg/g dried biomass) at 150°C. Carbohydrate yield deceased to about 50 mg/g dried biomass at 200°C, but protein yield was much higher on the contrary. The possible explanation is that peptide bond is more stable than glucosidal bond. It needs more energy to hydrolyse protein, and higher temperature is advantage. Maillard reaction consumes much carbohydrate and relatively little protein. Then protein yield became much greater than carbohydrate yield. Although Maillard reaction intensifies with higher temperature, it consumes more carbohydrate than protein[29]. Protein was consumed less than accumulated at 200°C. It was seemed that the hydrothermal initial pressure seemed no promotion to protein at temperature of 100°C. Liquid water seldom gasified at temperature of 100°C, because the pressure inside reactor was the same with saturation pressure of 100°C without adding nitrogen gas. But initial pressure of 4 MPa promoted approximate 60 mg protein/g dried biomass at 150°C and 200°C. It could be concluded that protein yield increased mostly (from 85.95 to 211.8 mg/g dried biomass) at 150°C with retention time varied from 0 to 120 min, it only increased 19.04 mg/g dried biomass at 100°C, and 7.77 mg/g dried biomass at 200°C from Figure 2b and Table 2. The explanation was that protein was hard to hydrolysis and release at low temperature, even if retention time was long. Long retention time not only resulted to more protein releasing, but also caused more protein consumed by Maillard reaction at high temperature.



a. Effects of hydrothermal temperature and initial pressure with retention time 60 min



b. Effects of hydrothermal temperature and retention time with initial pressure 2 MPa

Figure 2 Surface plots for protein yield during concentrated wet *Chlorella vulgaris* biomass hydrothermal hydrolysis

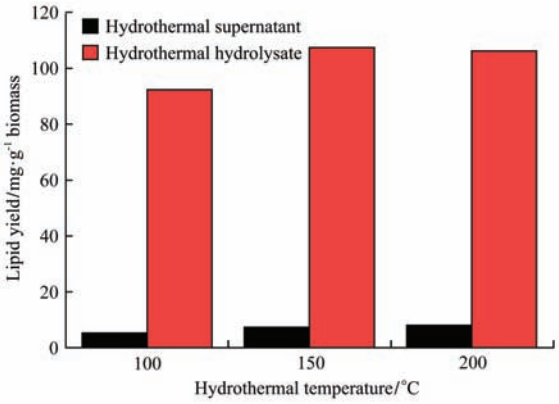
Both carbohydrate and protein are soluble substance. They can easily be released from broken cells to water and then be used for biogas fermentation. However, the other main component of microalgae biomass, lipid hardly dissolved in water, it still mainly left in the microalgae residue after hydrothermal hydrolysis. In order to use microalgae biomass at the greatest degree, lipid in the residue was extracted after medium-low temperature hydrothermal hydrolysis for biodiesel production.

3.3 Lipid extraction after medium-low temperature hydrothermal hydrolysis

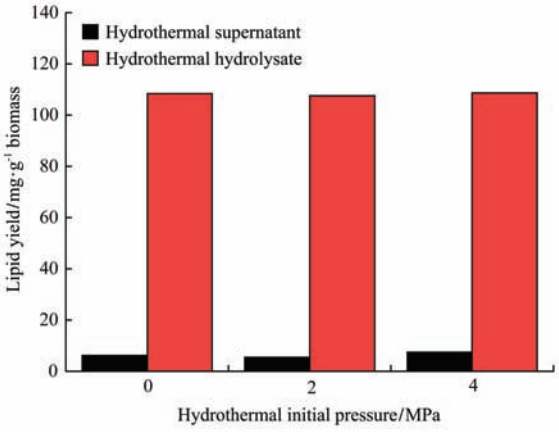
According to similar dissolve mutually theory, lipid should be only dissolved into nonpolar solvents such as hexane. However, hexane can hardly access into wet microalgae because of the water layer surround cell wall. Methanol can easily break the water layer, but it cannot damage cell wall efficiently. As shown in Figure 3, with mixed hexane and methanol adding in, lipid yield extracted from concentrated wet *Chlorella vulgaris* hydrothermal hydrolysate (with microalgae residual) was up to 123.3 mg/g dried biomass (approximately 67.69% of microalgae lipid was extracted out). Conversely, the maximum lipid yield extracted from hydrothermal supernatant without microalgae residual was only 8.5 mg/g dried biomass (only 4.66% of microalgae lipid was extracted out). That indicated carbohydrate and protein could be released out from damaged or broken cells easier for their water solubility, but lipid hardly released out itself without the assist of extractant. That made lipid yield extracted from hydrothermal hydrolysate with microalgae residual was almost 13 times higher than from hydrothermal supernatant without microalgae residual. It meant that lipid mainly distributed in microalgae residual after pretreatment by medium-low temperature hydrothermal hydrolysis but seldom in hydrothermal supernatant. One probable explanation for the phenomenon is that lipid dissolves hardly in water so that little lipid distributed in liquid. The other is that microalgae cell wall was not broken completely by medium-low temperature hydrothermal hydrolysis, so that lipid was bared to releasing out.

Figure 3a shows effect of hydrothermal prereatment temperature on lipid extraction and with 2 MPa initial pressure for 60 min on lipid yield extracted from hydrolysed *Chlorella vulgaris* biomass supernatant. It shows that lipid yield extracted from hydrothermal hydrolysate increased obviously 93.31-107.4 mg/g dried biomass with the increasing of hydrothermal temperature from 100°C to 150°C. But lipid yield was almost stabled at 106.2 mg/g dried biomass when the hydrothermal temperature further increased to 200°C. That mainly because the low temperature hydrothermal hydrolysis (<150°C) could already cause damage to microalgae cells, lipid could extracted out by hexane. But this medium temperature hydrothermal hydrolysis (150°C-200°C) could not cause microalgae cells completely broken and the lipid could not extract out all. That may be why lipid yield could not increase with the increasing of hydrothermal pretreatment temperature from 150°C to 200°C. Figure 3b shows the effect of hydrothermal pretreatment initial pressure on lipid extraction from hydrolysed *Chlorella vulgaris* biomass supernatant under a temperature of 150°C for 60 min. It indicated that initial pressure had no influence on lipid yield, it was similar to hydrothermal liquefaction[30]. Figure 3c shows effect of hydrothermal pretreatment retention time on lipid extraction from hydrolysed *Chlorella vulgaris* biomass supernatant under 2 MPa initial pressure and temperature of 150°C. It was seemed that more lipid was extracted from hydrolysed *Chlorella vulgaris* biomass supernatant with increasing retention time. It is mainly because longer retention time resulted in more cells' wall damaged.

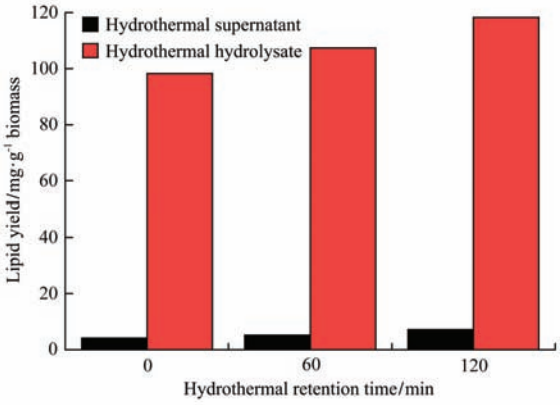
By medium-low temperature hydrothermal hydrolysis, carbohydrate and protein released out of microalgae cells into water, but lipid still left in microalgae cells for its water insolubility. From this aspect, lipid can be separated from carbohydrate and protein by sedimentation or centrifugation. The separated lipid, carbohydrate and protein then could be used to produce biodiesel and biogas, respectively. By this way, it can realize the full use of microalgae biomass.



a. Effects of hydrothermal pretreatment temperature on lipid extraction



b. Effects of hydrothermal pretreatment initial pressure on lipid extraction



c. Effects of hydrothermal pretreatment retention time on lipid extraction

Figure 3 Comparison of lipid extracted from hydrolysed *Chlorella vulgaris* biomass supernatant (without residual) and hydrolysate (with residual) with mixed hexane and methanol

4 Conclusions

Hydrothermal hydrolysis kinetic models describing the coupled effects of temperature, initial pressure and retention time on hydrothermal hydrolysis process was developed. The models predicted that the maximum carbohydrate yield would reach 335.5 mg/g under initial pressure of 3.44 MPa at temperature of 146.4°C for 116.08 min, and maximum protein yield would be 321.7 mg/g under initial pressure of 2.77 MPa at hydrothermal hydrolysis temperature of 196.4°C for 56.74 min. Lipid was mainly distributed in hydrothermal microalgae residual but seldom in supernatant, while carbohydrate and protein were in supernatant. Thus, lipid could be separated from carbohydrate and protein by gravity or centrifugation. Both carbohydrate and protein can be used to produce biogas by fermentation, and lipid can be used to make up of liquid fuel (such as biodiesel). In this respect, medium-low temperature hydrothermal hydrolysis pretreatment can maximize its utilization enhance microalgae biomass energy application.

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