

Effects of Low Temperature Hydrothermal Pretreatment on N Distribution and Pyrolysis Characteristics of Chlorella pyrenoidosa Residue with High Protein Content

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Abstract: The hydrothermal degradation characteristics of high-protein chlorella in low-temperature hydrothermal pretreatment were studied by hydrothermal kettle. The product yield, elemental composition, energy recovery and key elements C and N of 125-200 °C were investigated. The results show that a large amount of C and N elements are enriched in the aqueous phase, and increasing the temperature (>175 °C) is beneficial to the deamination reaction and thus the relative content of NH3 in the aqueous phase. As the pretreatment temperature increases, the distribution of N in the oil phase product increases gradually and increases rapidly after 175 °C; the solid phase yield and energy recovery rate gradually decrease, but at the same time, its N/C and O/C also decrease, indicating Hydrothermal pretreatment is a upgrading process. The functional group structure and thermal degradation characteristics of the raw materials and algae residue were analyzed by FT-IR, XPS, TG-FTIR-MS and Py-GC-MS. The results show that the hydrothermal pretreatment process changes the relative content of C-N and C-O decreases. In addition to protein-N and Quaternary amine-N, a small amount of pyridine-N appeared in the algal residue after pretreatment. The content and structural changes of the functional components of the solid algae residue reduce the release of NH3 and HCN in the thermogravimetric process and the content of the N-containing heterocyclic compound in the rapid pyrolysis product.

Keywords: High Protein Chlorella; Low Temperature Hydrothermal Pretreatment; Functional Group Structure; Thermal Degradation Characteristics

In order to alleviate the excessive dependence on fossil energy and the serious environmental pollution caused by it, countries around the world are working to develop and utilize various renewable energy sources including biomass energy. The significant advantages of seaweed, such as rapid growth, no occupation of cultivated land, effective fixation of CO2, and absorption of N and P nutrients in water bodies, are considered to be key development targets for future energy. The fuel obtained from seaweed is called the third generation biofuel and is the most ideal oil substitute in the 21st century ^[1, 2]. At present, there are two main ways to prepare liquid fuel using seaweed: one is to prepare biodiesel by extracting oil ester transesterification. The method is suitable for algae with high oil content; the second is to prepare bio-oil by thermochemical conversion method (hydrothermal liquefaction, pyrolysis liquefaction, etc.), fully utilize raw material composition, and has high conversion efficiency.

At present, many countries in the world have carried out extensive research on the preparation of seaweed bio-oil by thermochemical conversion method ^[3, 4], and investigated external factors such as reaction conditions (temperature, reaction time, catalyst, etc.) and internal components ^[5-8] and composition. Factors ^[9,10] have an effect on the yield and composition of bio-oil. It is found that in addition to oil, the sugars and proteins in the raw materials can be effectively converted into bio-oil, and the prepared algae bio-oil has a higher calorific value than lignocellulosic fiber. Bio-oils (20-25 MJ/kg), close to the calorific value of crude oil (42 MJ/kg) ^[11]. However, it should be specially pointed out that compared with N (0.1%) in lignocellulosic biomass, the N content in algal biomass is relatively high (5%-9%), resulting in the N content in the prepared bio-oil. Higher (2.7%-9.1%) ^[1,12,13], which will cause a large amount of NOx emissions in

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subsequent combustion utilization. Further component analysis found that N in algae bio-oil mainly exists in the form of N-containing heterocyclic compounds (pyridine, hydrazine, etc.) and non-heterocyclic amine compounds (such as amides, fatty amines, etc.) ^[10,14]; These N-containing compounds will easily cause poisoning of the catalyst during the subsequent upgrading process, and the degradation effect of the catalyst on the N heterocyclic compound is extremely poor ^[15].

The N-containing compounds in bio-oil are mainly derived from the polymerization of protein degradation products with sugar or oil degradation products ^[16]. Therefore, in order to effectively reduce the N content in the algae bio-oil, N in the protein can be concentrated in the aqueous phase by low-temperature hydrothermal pretreatment, thereby preventing the formation of the N-containing compound from the source. Jazrawi et al ^[17] found that the two-step hydrothermal liquefaction method can reduce the N content of bio-oil from 7.5% to 3.4%; Du et al. ^[18] found that low-temperature hydrothermal pretreatment coupled pyrolysis. In the way of oil production, the type and content of N-containing compounds in bio-oil obtained after pretreatment are significantly lower than that of untreated bio-oil; Huang et al. ^[19] have shown that water temperature is low (150-225 ° C). Pretreatment can effectively remove 11%-61% of N in algae.

It can be seen that the low-temperature hydrothermal pretreatment method can effectively reduce the N content in bio-oil, but there are few reports on related aspects, especially the mechanism of pretreatment of algae biomass by lowtemperature hydrothermal is still unclear. In particular, there are few reports on the distribution characteristics of key elements C and N in each phase product and the influence of hydrothermal pretreatment on the chemical structure and thermal degradation characteristics of solid algal residue. In this study, chlorella was selected as the experimental object. The effect of hydrothermal pretreatment on the yield of each phase product was investigated. The distribution of key elements C and N in the products of each phase was analyzed. The pretreatment was mainly investigated. The effect on the chemical structure and thermal degradation characteristics of solid algae residue; in order to explore the degradation mechanism of high-protein algae in the process of low-temperature hydrothermal pretreatment, and provide basic data for efficient use of solid algae residue.

1. Experimental Part

1.1 Experimental Materials

Chlorella is from the trihydrate microalgae culture site (Foshan, Guangdong), dried, pulverized, sieved (200 mesh, \leq 75 µm) and stored in a desiccator for later use. Table 1 shows elemental analysis, industrial analysis and component analysis of chlorella. Among them, the ash and volatile content in industrial analysis refer to ASTM E1755-01 and E872-82, respectively, and the protein and lipid content in the components refer to Chinese national standard GB5009.5-2016 and GB5009.6-2016, respectively; element content and heat values were measured by a VarioEL Cube elemental analyzer (Elementaranalyse, Germany) and a WZR-1T-CII automatic calorimeter (Changsha Bente Instruments Co., China), respectively. As can be seen from Table 1, chlorella contains higher protein (42.77%, mass fraction) and N content (8.57%, mass fraction), which is significantly higher than the N content in conventional lignocellulosic biomass (0-1.0%) ^[20].

Table 1	Proximate,	component	and ultir	nate analy	ses of	Chlorella	(dry	basis)	
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Proximate analysis <i>w</i> /%		Component w/%			Ultimate analysis <i>w</i> /%					Atomic ratio			$Q_{\rm HHV}$ /	
V	\mathbf{FC}^*	A	protein	lipids	$\operatorname{carbohydrates}^*$	С	Н	0^{*}	Ν	S	H/C	N/C	0/C	(MJ•kg ⁻¹)
80.80	12.06	7.14	42.77	8.01	42.08	52.45	7.12	23.84	8.57	0.88	1.63	0.14	0.34	23.62

^{*} by difference

1.2 Hydrothermal Pretreatment Experiment

Accurately weigh 10g of dried chlorella (with an error of no more than ± 0.01 g), add it to a hydrothermal kettle (304 stainless steel, 250mL) together with 100mL of deionized water, and seal it into high-purity argon for 20min. Air in the net. Turn on the heating device to raise the temperature to the target temperature (125-200 ° C, temperature interval 12.5 ° C, temperature control accuracy ± 0.1 ° C) and keep it for 30 min (previous study [21, 22] shows that when the reaction time exceeds 30 min, the yield distribution of each phase does not change much with time. Therefore, the reaction time

is 30 min). After the reaction is finished, the hydrothermal kettle is cooled to room temperature, and the gas is heated to open the hydrothermal kettle; In order to reduce experimental errors, repeat the experiment 2 or 3 times at each temperature point.

1.3 Separation and Collection of Each Phase Product

Product separation and collection directly affect the distribution of each product. The specific process schematic is shown in Figure 1.



Fig. 1 schematic diagram of product separation and collection process

After washing the reaction with 100 mL of deionized water, the product was mixed. After centrifugation, the supernatant was collected. The above steps were repeated once, and the collected supernatants were mixed and made up to 500 mL, and 100 mL was dried at 55 ° C. Dry until the quality does not change, the resulting product is water solubleorganics (wso); the above separated solid product and a certain amount of isopropanol are mixed, ultrasonically shaken for 20 min, centrifuged, and the upper isopropanol phase is collected. It is evaporated to remove isopropanol at 55 ° C, and the obtained oil product is obtained. The solid phase product obtained by centrifugation is further dried at 105 ° C to a constant weight, and the obtained solid residue is solid.

The product yield of each phase is calculated from the formulas (1) to (4), wherein the gas phase product yield is obtained by subtractive subtraction.

$$w_{\rm wso} = \frac{m_{\rm wso} \times 5}{m_0} \times 100\%$$
 (1)

$$w_{\rm oil} = \frac{m_{\rm oil}}{m_0} \times 100\% \tag{2}$$

$$w_{\text{solid}} = \frac{m_{\text{solid}}}{m_0} \times 100\% \tag{3}$$

$$w_{\rm gas} = 1 - w_{\rm wso} - w_{\rm oil} - w_{\rm solid} \tag{4}$$

In the formula, wwso, whoi, wsolid and wgas represent the yields of the aqueous, oil, solid and gas phase products respectively; m0, mwso, moil and msolid are the initial mass of the raw materials and the aqueous phase, oil phase, solid after drying, the quality of algae residue, g, respectively.

1.4 Product Analysis and Characterization

1.4.4 Element Analysis and Energy Recovery Calculation

Quantitative analysis of N and C in the aqueous phase using a spectrophotometer (HACH, DR3900) and a total carbon analyzer (varioTOC, Elementar, Germany); solid algal residue and elemental analyzer (VarioEL III, Elementar, Germany). The oil phase product was subjected to elemental analysis.

The calorific value of the oil phase product (QHHV, oil) and the calorific value of the solid algal residue (QHHV, solid) are calculated by the formula (5), and the energy recovery rates of the two are calculated by the formulas (6) and (7), respectively.

$$Q_{\rm HHV} = 0.3491 \times C + 1.1783 \times H + 0.1005 \times S - 0.1034$$
$$\times O - 0.0151 \times N - 0.0211 \times A \qquad (5)$$
Energy recovery of oil = $\frac{Q_{\rm HHV,oil} \times w_{\rm oil}}{Q_{\rm HHV,algae}} \times 100\%$ (6)
Energy recovery of solid = $\frac{Q_{\rm HHV,solid} \times w_{\rm solid}}{Q_{\rm HHV,algae}} \times 100\%$ (7)

In the formula, C, H, S, O, N and A represent the content of each element and ash, respectively.

1.4.2 Microscopic Morphology and Functional Group Structure Characterization

The sample was characterized by scanning electron microscopy (SEM, Hitachi S-4800, Hitachi, Ltd., Japan) to characterize the microscopic morphology of algae raw materials and solid algae residue. Accelerating voltage: 2.0 kV, magnification: 4 .5k.

The chemical functional groups in the algae feedstock, solid algae residue and oil phase product were qualitatively analyzed by Fourier transform infrared spectroscopy (FT-IR, TENSOR27, Bruker Daltonics Inc., Germany); 4000-400 cm-1 scan, 64 scans.

The binding energy forms of C and N in algae feedstock and solid algae residue were characterized by X-ray photoelectron spectroscopy (XPS, ESCALAB250Xi, ThermoVGScientific, UK); the target source was monochromator Al target (1486.68eV), power 150W, the spot diameter is 500µm, and the electron levitation angle is 90°. Scanning in constant pass mode, with a pass energy of 30 eV and a 0.1 eV length.

1.4.3 Analysis of Thermochemical Properties

The thermogravimetric and functional group release characteristics of the raw materials and solid algal residue were compared by a synchronous thermal analysis-infrared spectroscopy-mass spectrometer (TG-FTIR-MS, STA449F3, NE-TZSCH, Germany). Each time we accurately weighed (40.0 ± 0.5) mg, the heating rate was 20°C/min; the infrared spectrum was set as above, the mass spectrometer scanned 200 times/min; the connecting pipe was insulated with heating tape to 250°C to avoid pyrolysis. The product condenses in the pipeline.

The pyrolysis product characteristics of the feedstock and solid algal residue were comparatively analyzed using a cracker-gas chromatography mass spectrometer (Py-GC/MS, cracker: CDS5000; Agilent 7890A-5975C, Agilent

Technologies Inc., American). Accurately weigh the raw material (0.50 ± 0.1) mg each time; pyrolysis temperature: 500°C, heating rate: 1000°C/s, residence time: 20s; column is HP-5MS weak polarity column. Heating procedure: it was first kept at 40 ° C for 4 min, then raised to 280 ° C at a temperature increase rate of 5 ° C / min, and finally retained at 280 ° C for 10 min.

2 Results and Discussion

2.1 Distribution Law of Product Yield of Each Phase

The yield distribution law of each phase of Chlorella in the pretreatment at 125-200 °C is shown in Fig. 2. It can be seen from Fig. 2 that as the pretreatment temperature is raised from 125 ° C to 200 ° C, the solid algae yield gradually decreases from 72.56% to 18.28% (mass fraction), which is consistent with the trend in the literature. ^[18] The main reason is that with the increase of pretreatment temperature, the macromolecular organic components in the raw materials are continuously degraded, and the degradation rate increases when the temperature is higher than 150 °C, which shows the solid algae yield above 150 °C. drop rapidly. Contrary to the trend of solid-phase algae residue, the oil phase and gas phase yields gradually increase at 125-200 °C, and the growth rate is obvious after 175 °C, reaching a maximum of 23.39% and 14.4% at 200 °C, respectively (mass fraction); the yield of gas, solid and oil phase products varies monotonically with temperature, and the yield of aqueous phase product increases first and then decreases with the increase of pretreatment temperature, and reaches a maximum value of 57.99 at 187.5 °C. % (mass fraction), when the temperature is further raised to 200 ° C, the yield of the aqueous phase product is greatly reduced and the oil phase yield is greatly increased; indicating that the hydrothermal pretreatment process first uses the macromolecular organic component in water as the reaction medium. The reaction system first decomposes into small molecules. As the temperature increases, the hydrothermal pretreatment process gradually changes to the hydrothermal liquefaction process, that is, the small molecules organic products dissolved in the aqueous phase further polymerize into macromolecules into the oil phase, improving the yield of oil phase product ^[23].



Figure 2 Yields of wso, oil, solid and gas from hydrothermal pretreatment of Chlorella at 125-200 °C

solid; . sol

2.2 Element C and N Distribution Law and Energy Recovery Rate

Table 2 shows the elemental analysis, calorific value and energy recovery of solid algae and oil phase products at *Progress in Energy & Fuels* | 5

different pretreatment temperatures.

Operating		Ultimate	e analys	sis <i>w 19</i>	6	Ash	Atomic ratio			$Q_{\rm HHV}$	Energy
condition	N	С	Н	\mathbf{S}	0*	w 1%	H/C	N/C	O/C	/(MJ•kg ⁻¹)	recovery $\eta/\%$
Temp. t /℃					soli	d obtained	l at holdi	ng time	of 30 min		
125	8.48	50.41	6.90	1.04	28.88	4.29	1.642	0.144	0.430	22.63	69.49
137.5	8.20	50.57	7.12	0.86	29.24	4.01	1.689	0.139	0.434	22.90	61.94
150	7.52	50.93	7.26	0.79	29.38	4.12	1.710	0.127	0.433	23.17	55.14
162.5	6.49	51.87	7.30	0.68	29.41	4.25	1.690	0.107	0.425	23.55	40.78
175	6.07	53.45	7.52	0.64	28.11	4.22	1.689	0.097	0.394	24.50	33.68
187.5	5.70	56.96	7.66	0.50	24.77	4.41	1.614	0.086	0.326	26.22	25.20
200	5.34	60.22	7.69	0.31	20.82	5.61	1.533	0.076	0.259	27.77	21.48
Temp. t /℃					oil	obtained	at holdin	g time o	f 30 min		
125	1.60	64.39	9.33	0.90	23.78	-	1.739	0.021	0.277	31.08	6.44
137.5	1.91	66.41	9.90	0.56	21.22		1.788	0.025	0.240	32.68	5.80
150	1.98	67.20	9.68	0.42	20.72	-	1.728	0.025	0.231	32.73	8.17
162.5	3.42	66.89	9.78	0.42	19.49	-	1.754	0.044	0.219	32.85	13.48
175	3.46	68.18	9.96	0.40	18.00	-	1.752	0.043	0.198	33.66	12.96
187.5	5.76	66.37	9.16	0.19	18.52	-	1.656	0.074	0.209	31.98	13.54
200	6.59	65.15	8.88	0.51	18.88	_	1.635	0.087	0.217	31.21	30.90

Table 2 Ultimate analysis, $Q_{\rm HHV}$ and energy recovery of oil and solids

* : by difference

It can be seen from Table 2 that as the pretreatment temperature is raised from 125 ° C to 200 ° C, the N content in the solid phase algae slag gradually decreases from 8.48% to 5.34%, while C and H are from 50.41%, respectively. And 6.90% increased to 60.22% and 7.69%; compared with N/C (0.144) and H/C (1.642) in chlorella raw materials, N in solid algae residue /C decreases and H/C remains almost unchanged (except 200 °C), indicating that the degree of hydrolysis of the N-containing component during hydrothermal pretreatment is higher than that of other components; in addition, the calorific value of solid algal residue and the calorific value of the raw material (23.62MJ/kg) is not much different and increases with the increase of pretreatment temperature; therefore, the hydrothermal pretreatment process is also a raw material upgrading process, and the pretreated solid algae residue can be used as a potential application. Biomass raw materials. The C and H of the oil phase products did not change much at 125-200 °C, and the O content gradually decreased with increasing temperature. The N content slowly increased from 1.60% to 3.46% at 125-175 °C, while at 175-200. °C rapidly increased from 3.46% to 6.59%; this is mainly because at lower temperatures, the protein is first hydrolyzed into small molecular products such as amino acids, and as the temperature is further increased, the small molecule nitrogen-containing compounds and sugars. The hydrolysis product of the oil and fat is polymerized to form an oil phase product containing N macromolecules, thereby increasing the N content of the oil phase product.

In addition, in the hydrothermal pretreatment process, as the pretreatment temperature increases, the energy recovery rate of solid algal residue gradually decreases from 69.49% to 21.48%, and the energy recovery rate of the oil phase product is 125 °C. The 6.44% of the time gradually increased to 13.54% at 187.5 °C, and quickly reached the highest value of 30.90% at 200 °C, indicating that the pretreatment process is an energy redistribution process. It should be specially pointed out that the sum of the energy recovery rates of the solid algae residue and the oil phase product decreases first and then increases with the increase of the pretreatment temperature, indicating that although further increasing the temperature helps to improve the energy recovery rate of the pretreatment process, it is also greatly increased. The N content of the oil phase product was increased (shown in Table 2). Therefore, when selecting the pretreatment temperature, factors such as energy recovery, oil phase and N content in the solid phase product should be considered.



Figure 3C and N distribution in the products of each phase and NH3-N in the aqueous phase account for N-total content

Figures 3(a) and (b) show the distribution of energetic element C and nutrient element N in each phase product at different pretreatment temperatures. It can be seen from Fig. 3 (a) and (b) that when the pretreatment temperature is not higher than 187.5 ° C, C and N are mainly concentrated in the solid algae residue and the aqueous phase product, when the temperature is further raised to 200 ° C More C and N enter the oil and gas phase products. As the temperature increased from 125 ° C to 187.5 ° C, the distribution of C and N in solid algae residue decreased from 69.74%, 71.80% to 24.66%, 15.10%, respectively. It is because as the temperature increases, the hydrolysis reaction of sugars, proteins and other components in the algae is intensified, and water-soluble compounds such as amino acids and reducing sugars are formed. These compounds are enriched in the aqueous phase $^{[24]}$, resulting in an aqueous phase. The C and N distributions are gradually increased $^{[25]}$ and reach a maximum of 50.50% and 63.00% at 187.5 °C, respectively; when the temperature continues to rise to 200 °C, C and N are in the aqueous phase. The amount of distribution is reduced, and the amount of distribution in the oil phase and the gas phase is significantly increased; this is mainly because: on the one hand, the amino acid enriched in the aqueous phase will be polymerized with reducing sugar or oil to form a macromolecular oil phase product. The amount of C and N in the oil phase product is increased; on the other hand, the intermediate hydrolysate such as amino acid further decarboxylates and deamination, and a small molecule gas phase product is formed, resulting in a significant distribution of C and N in the gas phase product increase.

In order to verify the deamination reaction in the hydrolysis process, the total nitrogen (N-total) and ammonia nitrogen (NH3-N) enriched in the aqueous phase were further quantitatively analyzed. Figure 3 (c) shows the aqueous phase at different pretreatment temperatures. Medium ammonia nitrogen accounts for the total nitrogen content. It can be seen from Fig. 3(c) that at a temperature not higher than 175 ° C, although the N enriched in the aqueous phase increases with temperature (see Fig. 3 (b)), the ammonia nitrogen ratio remains almost at the total nitrogen ratio. 4% is unchanged, indicating that the process of hydrolysis of protein into amino acid is mainly in this temperature range; when the temperature is raised to 187.5 ° C, the content of ammonia nitrogen in total nitrogen is increased from 3.20% to 9.91%. And further increased to 14.09% at 200 ° C, which indicates that the amino acid further deamination reaction at higher temperatures.

Therefore, considering the element content, distribution law and energy recovery rate of each phase product, it can be seen that in the hydrothermal pretreatment process, proper temperature increase is beneficial to more N enrichment in the *Progress in Energy & Fuels* | 7

water phase, but the temperature is too high. It will increase the N content of the oil phase product and reduce the energy recovery. For Chlorella, a suitable pretreatment temperature range is about 175 °C.

2.3 Analysis of Functional Group Structure and Thermal Degradation Characteristics of Solid **Algae Residue**

According to the previous analysis, hydrothermal pretreatment is a raw material upgrading process. Therefore, in order to use solid algae residue more effectively, it is necessary to understand the change of physical structure before and after hydrothermal. At the same time, its functional group structure and thermochemical properties were analyzed and characterized.

2.3.1 SEM Analysis

SEM photographs of the chlorella raw material and the pretreated pretreated algae residue are shown in Fig. 4.



(a) raw material

(c) 200 °C-solid

Figure 4 SEM photograph of raw materials and solid algae residue

It can be seen from Fig. 4 that after the raw material (a) is dried, the cells become dry and flat, and the cell structure is still intact. During the hydrothermal pretreatment, the cells rupture, and as the temperature increases, the cells aggregate and stick and the contour gradually disappeared, see Figure 4(b) and Figure 4(c). When the pretreatment temperature was 175 ° C, a large number of broken and compacted cells were clearly observed, indicating that at lower temperatures (< $175 \,^{\circ}$ C), the cells ruptured and aggregated under hydrothermal conditions, as the temperature continued to rise. At the time, the aggregation was aggravated and accompanied by a series of reactions, the cell outline gradually disappeared, and no obvious cell outline could be observed at 200 °C. From the SEM analysis, the structure of algae cells changed greatly before and after hydrothermal pretreatment, and the change of chemical functional groups was further analyzed by FT-IR and XPS.

2.3.2 Functional Group Structure Characterization

Figure 5 is a FT-IR spectrum of the raw material and solid algal residue.



Figure 5: Infrared spectrum of raw materials and solid algae residue

In Fig. 5, 3400 cm-1 is a hydroxy O-H of a saccharide and a N-H of a peptide bond; 2921 and 2853 cm-1 are a methyl group in a fat or a saccharide and a C-H on a methylene branch; 1647 cm-1 is a superposition of one or several double bonds C (C=C, C=N, C=O); 1537 and 1247 cm-1 are respectively CHN bending vibration and C-N of secondary amide (R-CONH-R) Telescopic vibration; 1450-1350cm-1 contains bending vibrations of three structures: O-H, N-H and C-H; 1158 and 1033cm-1 are C-O of esters and saccharides and C-C of organics, and skeleton respectively^[26-28]. The corresponding representative substances at 1537 and 1247cm-1 are proteins, and the decrease of the infrared peak intensity at these two places indicates that the protein gradually hydrothermally forms amino acids dissolved in water during hydrothermal process, and the solubility increases with the increase of the pretreatment temperature. Comparing the infrared spectra of raw materials and solid algae residue, the effect of functional group changes before and after hydrothermal process. The intensity of the infrared peak is lowered. Similarly, some groups with poor thermal stability (such as C=C, C=O) will undergo addition or cleavage, which also causes a decrease in the intensity of the infrared absorption peak.

It can be seen from the infrared spectrum that the intensity of some functional groups of the solid algae residue after hydrothermal pretreatment is somewhat weakened, and the C and N functional groups are further characterized by XPS, and the peak fitting map is shown in Fig. 6.



Figure 6 shows the XPS (C1s, N1s) spectrum of raw materials and solid algae residue

According to previous studies [29-31], the functional groups of C can be divided into five types: C1 (C-C and C-H), *Progress in Energy & Fuels* | 9

EB is 284.8 eV; C2 (C-N), EB is 285.8 eV; C3 (C-O), EB is 286.6 eV; C4 (C=O), EB is 287.8 eV; C5 (O-C=O), EB is 288.8 eV; N functional groups can be divided into three types: N1 (protein-N), EB was 399.7 eV; N2 (quaternary amine-N), EB was 401.4 eV; N3 (pyridine-N), EB was 398.4 eV, and the relative content of each peak area is shown in Table 2. After hydrothermal pretreatment, the relative contents of C-C and C-H in the solid algae residue increased significantly, compared with the raw materials (39.82%), which were 58.37% (175 °C) and 70.41%, respectively. 200 °C), similarly, the relative content of C-N, C-O and C = O have a certain degree of reduction, this change indicates that amides, alcohols and aldehydes and ketones in Chlorella are more susceptible to hydrolysis into other phases, the content of N, O functional groups in the solid algae residue is reduced. The decrease in the relative content of O-C=O is mainly due to the enrichment of the oil in the chlorella in the oil phase product. Comparing the two pretreatment temperatures at 175 and 200 °C, it was found that the higher the pretreatment temperature, the more obvious the change of C relative content of different binding energy forms. It can be seen from the N spectrum that N in chlorella is mainly in the form of protein-N (N1, 96.17%), and also contains a small amount of quaternary amines - N (N2, 3.83%), which is mainly a quaternary amine compound such as betaine contained in algae [32], which is relatively stable during hydrothermal pretreatment. After hydrothermal pretreatment, the relative content of protein-N in the solid algae residue is reduced, mainly because the peptide bond of the protein is broken during the pretreatment to form a small molecule of amino acid enriched in the aqueous phase; except for protein-N and amine-N, a part of N in the solid algae residue (about 5.9%) exists in the form of pyridine-N, mainly because the Maillard reaction of amino acid and reducing sugar occurs further at this pretreatment temperature, pyridine-N [33] was produced.

Table 3 Relative peak area of C and N by XPS analysis

Peak –	Relative peak area A/%												
	C ₁	C_2	C ₃	C_4	C ₅	\mathbf{N}_{1}	N_2	N 3					
Raw material	39.82	23.92	21.66	9.47	5.13	96.17	3.83						
175 ℃-solid	58.37	18.22	13.58	6.97	2.87	90.78	3.28	5.94					
200 ℃-solid	70.41	15.81	6.75	5.50	1.54	88.80	5.30	5.90					

2.3.3 Thermal Weight Loss and Product Release Characteristics

The thermogravimetric process and product release characteristics of chlorella raw materials and solid algae residue after pretreatment at 175 and 200 °C were studied by TG-FTIR-MS. The results are shown in Fig. 7-9.



Figure 7 Thermogravimetric characteristics of chlorella and solid algal residue

It can be seen from Fig. 7 that the thermal weight loss of chlorella and solid algal residue mainly occurs at 200-550 ° C, and the weight loss process can be roughly divided into two stages. The first stage (200-390 ° C) is mainly the decomposition of polysaccharides and amino acids such as starch ^[34] and the removal of polyacyl groups ^[35]. Compared with the weight loss rate of raw materials (49.00%), the weight loss rate of solid algae residue gradually decreased from 46.95% to 38.34% as the pretreatment temperature increased from 175 °C to 200 °C; the first weight loss The peak appeared near 310 °C, and the peaks were close, which were 0.44%/°C, 0.47%/°C, and 0.41%/°C, respectively. This indicates that the proteins and polysaccharides in the algae are gradually hydrolyzed, and the amino acids and small molecular sugars are

dissolved in the water phase, while the relative content of the two kinds of substances in the solid algae residue causes the weight loss of the first weight loss peak to gradually decrease. The weight loss in the second stage (390-550 °C) is mainly the further pyrolysis process of polysaccharides and amine intermediates after dehydration. The hydrocarbon groups in the branches will be removed and the main chain C-C skeleton will be removed. Cyclization produces some small molecular hydrocarbon gases ^[36], and amine intermediates deamination to form NH3. Compared with the weight loss rate of raw materials (17.40%), the weight loss rate of solid algae increased from 23.49% to 33.36% as the pretreatment temperature increased from 175 °C to 200 °C; the second weight loss peak appeared. At 450 °C, the weight loss peak of solid algae residue is significantly larger than that of chlorella, and with the increase of pretreatment temperature, the peak value of the second weight loss peak increases from 0.18%/°C of raw material to 0.42 at 200°C. %/°C. The change of the second weight loss peak indicates that hydrothermal pretreatment removes water-soluble groups, especially the removal of O-containing groups, which increases the relative contents of C and H, making hydrocarbon small molecules at this stage. Gas release increases. These changes indicate that the hydrothermal pretreatment process changes the thermal degradation characteristics of algae to some extent.

Combined with the thermogravimetric curve of Fig. 7, the infrared spectrum and the release rule of functional groups under the temperature loss characteristic temperature are analyzed, as shown in Fig. 8 and Fig. 9.



Figure 8 FT-IR spectrum of chlorella and solid algal residue at characteristic pyrolysis temperature

It can be seen from Fig. 8 and Fig. 9 that the wave number is corresponding to the gaseous H2O molecule at 3750 cm-1, which is mainly derived from the dehydration reaction process of some algae macromolecules. It can be seen from the figure that the dehydration reaction mainly occurs when the early temperature is low, at 390. No release of gaseous H2O molecules was observed on the FT-IR spectrum after °C. Wavenumbers 3013 and 2958 cm-1 are characteristic peaks of CH4 and hydrocarbon functional groups (-CH3-, -CH2-), and their release patterns are shown in Fig. 9(a) and Fig. 9(e). The law is similar, mainly released at 400-600 °C, which is derived from the release of branched hydrocarbon groups at high temperature, compared with the change of raw materials and pretreated algae slag at 175 and 200 °C, and found that with the increase of pretreatment temperature, hydrocarbons. The increase in the amount of gas released indicates that the pretreatment process increases the content of hydrocarbon groups in the algae residue, which is consistent with the results of the previous XPS analysis of C spectrum, and is also one of the reasons for the change in weight loss rate in the second

stage.

The absorption peaks at wavenumbers 2358 and 2342 cm-1 represent CO2, which is mainly derived from the cleavage and reforming of carbonyl (C=O) and carboxyl (-COOH) ^[37]. The release law of CO2 is shown in Figure 9(c). The main release temperature is 200-500 ° C, and the absorbance peaks near 310 ° C. The peak of the algae residue after the pretreatment of the raw material and 175 ° C is similar, while the peak width is slightly narrowed, and the peak of algae is reduced after pretreatment at 200 ° C. The peak position was shifted back to 350 ° C, after which the curve coincided with the 175 ° C algal residue curve. This change indicates that the carbonyl and carboxyl groups are more stable during the pretreatment process before 175 ° C, while the pretreatment temperature continues to increase, the decarbonylation and decarboxylation reactions are intensified, and the content of these groups is significantly reduced at 200 ° C. The infrared absorption peak at wavenumber of 1750 cm-1 corresponds to the stretching vibration of C=O, indicating the release of some small molecule carboxylic acids and aldehydes and ketone gases, which are derived from the decomposition of acetyl groups ^[34], and the release law is shown in Fig. 9 (f), only one absorption peak appears in the pyrolysis of the raw material, which is at 200-500 ° C, and two absorption peaks appear in the algal residue after pretreatment, while the first absorption peak showed no significant change.

The wave number is NH3 and HCN^[38] at 961 and 718 cm-1, respectively. It is mainly derived from the pyrolysis of amino acids. The release rules of the two are similar with temperature, as shown in Fig. 9(b) and Fig. 9(d), respectively. It can be seen from Fig. 9(b) and Fig. 9(d) that only one absorption peak appears in the chlorella raw material, and the peak appears at 310 °C. This absorption peak gradually decreases with the increase of the pretreatment temperature, and new at 475 °C. An absorption peak appears, the peak value of which increases with the increase of the pretreatment temperature; this may be because the proteins in the chlorella cells form a polypeptide such as cyclic amide in the hydro-thermal pretreatment process, and these polypeptides are first cleaved into HCN at high temperature. And HCNO^[39], while HNCO will further decompose to form HCN and CO, forming a second HCN release peak.



Fig. 9 Infrared spectrum of product release characteristics of chlorella and solid algal residue

At the same time, the product release law was analyzed by online mass spectrometry. The molecular release patterns of relative molecular masses of 16, 17, 27 and 44 are shown in Fig. 10(a)-10(d). 10(a)-10(d), CH4 (m/z=16), NH3 (m/z=17), HCN (m/z=27), and CO2 (m/z=44). The variation of the gas on the mass spectrum is basically the same as that on the infrared spectrum, which also verifies the release law on the infrared spectrum. The gas molecule release law of m/z=30 is shown in Fig. 10(e), and its release is at 200-550 °C. The chlorella raw material shows a peak near 280 °C, and the infrared spectrum shows the gas released at this temperature. It should be HCHO, and the gases released at 400-500 °C are CH3CH3 and HCHO. The gas molecule release law of m/z=46 is shown in Fig. 10(f), and its release is at 200-400 °C, and a peak appears at around 250 and 350 °C, respectively, and the previous peak increases with the pretreatment temperature. Gradually decreasing, the latter peak gradually increases, which is derived from gas small molecules with C=O functional groups, such as CH3COCH3 and HCOOH, which are release laws of some small molecular substances were determined, which indicated that the release of these substances was accompanied by the whole thermogravimetric process, which was mainly caused by the fragmentation of branched groups and the further cracking process of macromolecular compounds.



Figure 10 Mass spectrum of product release characteristics of chlorella and solid algal residue

2.3.4 Py-GC/MS Analysis

The product distribution and relative content of chlorella raw materials and solid algae residue were further analyzed by Py-GC/MS, as shown in Figure 11. As can be seen from Fig. 11, the types of pyrolysis products before and after pretreatment have little change, and all contain hydrocarbons, acid esters, aldehydes and ketones, and N-containing compounds, but the relative contents of these compounds are largely different. As the pretreatment temperature increases, the

content of aliphatic hydrocarbons in the pyrolysis product increases from 15.37% at the time of the feedstock to 31.18% at 200 °C solid algae residue, which is related to the hydrocarbon functional group in the previous TG-FITR. The results of high release were consistent. Among them, the most abundant substance was hexadecene; while the content of aromatic hydrocarbons did not change much, indicating that aromatic organic compounds were stable during pretreatment. The Ncontaining compound is derived from the pyrolysis of proteins in algae and is further divided into N-containing heterocyclic compounds, amides and nitriles according to chemical structure [35, 39], wherein the N-containing heterocyclic compounds are mainly due to rapid pyrolysis cyclization of amino acids. The monocyclic and polycyclic N-containing compounds (such as ruthenium and pyrrole) are formed [40]. As the pretreatment temperature increases, the content of the Ncontaining heterocyclic compound decreases from 16.94% to 8.95% of chlorella (175 ° C) and 6.85% (200 ° C), indicating that hydrothermal pretreatment can effectively reduce the formation of N-containing heterocyclic compounds in pyrolysis products, which is conducive to further upgrading of pyrolysis products; in rapid pyrolysis, the nitrile is mainly derived from the secondary reaction between tar and cyclic amide [39], and its content decreases with the increase of pretreatment temperature, indicating the secondary reaction reduced during the pyrolysis of algae after pretreatment. Fatty amide is mainly derived from the reaction between fatty acid and amino acid [41], and after pretreatment, the content of fatty amide is slightly reduced (175 $^{\circ}$ C) and further increases with the increase of pretreatment temperature (200 $^{\circ}$ C). In contrast, fatty acid content mainly includes hexadecanoic acid and oleic acid, and mainly derived from algal lipid pyrolysis [42].



Figure 11 Distribution of rapid cracking products at 500 °C of chlorella and solid algae residue

The oxygen-containing heterocyclic compounds are mainly furans, and some of the aldehydes and ketones are derived from the pyrolysis of polysaccharides in algae ^[43]. It can be found from Fig. 11 that the aldehydes and ketones in the pyrolysis products of the algae after pretreatment are the content of O-containing heterocyclic compounds and is higher than that of raw materials, and further changes with the composition of N-containing compounds, indicating that during hydrothermal pretreatment, proteins are more easily hydrolyzed than polysaccharides, and the relative content of polysaccharides in algal residue increases after pretreatment; The treatment temperature was raised from 175 to 200 ° C, and the relative content of aldehydes and ketones in the pyrolysis product decreased, indicating that more sugars were 14 | Progress in Energy & Fuels

hydrolyzed at 200 ° C, resulting in a decrease in the sugar content of the solid algae residue.

3. Conclusion

Low temperature hydrothermal pretreatment can effectively improve the distribution of N elements in the products of each phase. At 125-200 °C, the pretreatment temperature can effectively enrich the N element in the water phase. When the temperature is higher than 175 °C, the deamination reaction will increase and the NH3-N in the water phase will increase significantly. The oil phase product yield and the energy recovery rate increases with the increase of temperature, but at the same time, the N distribution in bio-oil also increases continuously and increases rapidly after 187.5 °C; the solid phase product yield and energy recovery rate decrease with the pretreatment temperature, but at the same time N/C and O/C also decrease, indicating that the degree of hydrolysis of the N and O components in the pretreatment process is higher than that of the C component. Therefore, the hydrothermal pretreatment process is a raw material upgrading process.

Solid algal residue can be used as a good biomass material. Hydrothermal pretreatment changed the relative content of different functional groups of C and N. In the process of thermogravimetry, the NH3 and HCN released by algae after pretreatment were significantly reduced, and the content of N-containing heterocyclic compounds formed under rapid pyrolysis was significantly reduced. It is beneficial to the subsequent thermal conversion utilization of solid algae residue.

The pretreatment temperature should be considered in consideration of factors such as yield, composition and energy recovery. In this experiment, a reasonable pretreatment temperature is 175-190 °C.

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