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Valorisation of microalgae residues after lipid extraction: pyrolysis characteristics for biofuel production

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Abstract

As a promising source of renewable energy, biofuel from microalgae pyrolysis is seen as a competitive alternative to fossil fuels. However, currently, the widely applied pre-treatment process of lipid extraction results in large amounts of microalgae residues, which though with energy potential, being considered as process wastes and ignored of its re-utilization potential. In this study, a new workflow of biofuel generation from microalgae biomass through lipid extraction and pyrolysis of defatted microalgae residues was proposed and assessed. The effects of lipid extraction and pyrolysis temperature (350-750℃) on pyrolysis products were investigated, and pyrolysis pathways were postulated. To address the twin goals of lowering emission of pollutants and elevating energy products, an optimal pyrolysis temperature of 650℃ was suggested. After extraction of lipids, the relative contents of valuable products (aromatic, aliphatic hydrocarbons and fatty acids) and some harmful by-products, e.g., PAHs, significantly reduced, while other harmful substrates, e.g., nitrogen-compounds increased.

Mechanistic investigations indicated that pyrolysis of proteins without the presence of lipids could promote higher production of nitrogen-containing organics and aromatics. These results reveal the effects of lipid extraction and variation of temperature on microalgal pyrolysis, and also provide a basis for full utilization of microalgae as an aid to alleviate many fossil energy problems.

Keywords*:* Bioenergy; pyrolysis pathways; net-zero emission; microalgae technology; circular economy

1. Introduction

Increasing global fossil fuel consumption has led to the serious challenges of energy shortage and climate change [1,2]. Consequently, investigations into novel clean and sustainable energy sources are urgently required. Currently, international efforts are being conducted in order to attain net-zero emission targets by 2050 [3]. To date, broad types of renewable energy sources, such as wind, geothermal, solar, biogas, and biofuel, have been developed and deployed [4-6]. Among them, microalgae have been recognised as being a source of the third generation of biofuel, and have attracted widespread attention due to advantages of fast growth rate, high lipid content, small footprint, and the additional functionality of CO_2 sequestration [7]. Regarding the increasing intensive global warming, microalgae serve as a significant role for carbon neutralization and conversion towards divers products [8]. The nutrients for microalgal growth can also be supplied from wastewater, which is in plentiful supply [9]. Thus, sustainable microalgae technology for renewable energy, i.e. biofuel, production would also contribute to the much-desired circular economy [10].

Biofuel derived from microalgae could provide similar physical, chemical and operational characteristics as diesel, indicating the potential for using microalgal biofuel as an alternative clean energy [11,12]. Different techniques have hitherto been used to convert biomass to biofuel, including biochemical and thermochemical processes [13], although thermochemical conversions, such as pyrolysis, are much more rapid than

biochemical conversions, and have been commonly used to promote biofuel generation from raw microalgal biomass [14,15]. Recent studies have demonstrated that the direct extraction of lipids from microalgae through microwave assisted extraction, by the Kochert method, Soxhlet extraction, or ultrasonic extraction would result in the production of high quality biofuels [16]. Moreover, after removal of the lipids and other extractables from microalgal biomass, approximately half would remain as residues [17], because the lipid content normally contributes up to 55% of the total biomass [14]. Such residues have been re-used as hydrogen-rich feedstock for syngas production [18], soil amendment for agricultural production [19,20], and feed materials for biogas production through anaerobic digestion [21]. Nevertheless, the defatted microalgae residues, mainly consisting of protein and carbohydrate, still provide valuable biofuel potential, which, to date, has been insufficiently investigated.

In order to achieve complete consumption of microalgal biomass, the residues, after conversion of lipids into biodiesel, can be converted into bio-oil through pyrolysis [22]. Theoretically, pyrolysis of defatted microalgae residues requires much less energy input compared with raw biomass due to its less complex composition [23]. A previous, although limited, study has demonstrated that the amount of bio-oil generated from defatted *Scenedesmus* residues was only 7% lower compared with that directly produced from the raw algal biomass [24]. In view of entire life cycle, pyrolysis of defatted microalgae together with lipid extraction could contribute to a higher total oil yield than direct microalgal pyrolysis (43% increase of yield) [25]. However, most of the relevant investigations have only focused on the pyrolysis of microalgal residues regarding catalyst-assisted process [26]. However, the effects of pyrolysis conditions, e.g. temperatures and time, on biofuel generation from such defatted microalgal residues have not been systematically investigated. Assessments of relevant pyrolytic products, pathways of by-product generation, and the potential risks of producing harmful compounds would also be crucial to understand the underpinning mechanisms.

This study proposes and evaluates a new workflow for biofuel generation from microalgal biomass, which consists of biofuel generation from 1) extracted microalgal lipids and followed by 2) defatted microalgal residues through pyrolysis. As the first process has been sufficiently investigated [16], the current study focuses on the latter aspect. *Desmodesmus sp*., a chlorophyte alga, was selected as a model species and cultivated in anaerobic digestion effluent. The effects of different pyrolytic temperatures (350 ^{o}C-750 ^{o}C) on lipid extraction efficiency, biofuel production pathway, and potential harmful products generation assessed. This study aims at contributing to a systematic and comprehensive kinetic investigation for the pyrolysis of defatted microalgae, meanwhile, could provide alternative for safe pyrolysis product.

2. Materials and methods

2.1 Microalgae cultivation

Microalgae, *Desmodesmus sp*., were obtained from a local river in Liangxiang, Beijing. The algae was pre-cultured until reaching the exponential phase (OD_{680} of 0.14), and then inoculated under sterile conditions into a cultivation medium consisting of an anaerobically-digested effluent (ratio of 10%, v/v) [27], collected from a pig farm in Fangshan District, Beijing, China. The concentrations of ammonium nitrogen (NH_4^+-N), total nitrogen (TN) and phosphate phosphorus (PO_4^{3-}-P) were 720 ± 6, 792 ± 4 and 33 ± 0.1 mg L^{-1}, respectively. The concentrations of nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) were below 0.5 mg L^{-1} [28]. The cultivation was conducted under lighting conditions of 6000±100 lux, dark and illumination ratio of 10:14, and temperature of 25±1 ℃. After 14 days, samples were harvested by centrifugation at 10000 rpm for 15 min and dried by vacuum freeze drying (24h, -45℃).

2.2 Sample preparation and component analysis

The lipids from the raw microalgae were extracted by a solvent mixture (chloroform:methanol:water; 1:2:0.8, v/v) at 25 ℃ [29]. After vortexing, samples were left to stand until layers were formed. The lower phase (chloroform layer) was then removed and placed in a rotary evaporator for vacuum evaporation at 60℃ in order to

harvest the lipids. The upper layer containing the defatted residues was collected for further analysis.

The elemental compositions of raw and defatted microalgae, including carbon, hydrogen, nitrogen and sulfur, were determined by elemental analyzer (Vario EL, Elementar, Germany), using Sulfanilamide ($C_6H_8N_2O_2S$, molecular weight 172.22) as a standard sample to perform the instrumental calibration. The results of elemental analyses were then used to further calculate the protein content (wt %) and high heating value (HHV) of samples by equations (1-3) as noted in a previous study [30].

$$\text{Protein content} = \text{N} \times 6.25 \quad (1)$$

$$\text{HHV(OLS)} = 1.87\text{C}^2 - 144\text{C} - 2082\text{H} + 63.8\text{C} \times \text{H} + 129\text{N} + 20{,}147 \quad (2)$$

$$\text{HHV(PLS)} = 5.22\text{C}^2 - 319\text{C} - 1674\text{H} + 38.6\text{C} \times \text{H} + 133\text{N} + 21{,}028 \quad (3)$$

where, C, H and N represent the content of carbon, hydrogen and nitrogen elements, respectively, HHV represents higher heating values. Equations (2) and (3) were derived from regression analysis of ordinary least squares (OLS) and partial least squares (PLS) of high heating values for each sample.

The mean value of HHV (MJ kg^{-1}) was calculated according to equation (4):

$$\text{HHV} = \frac{\text{HHV(OLS)} + \text{HHV(PLS)}}{2}$$
$$= (3.55\text{C}^2 - 232\text{C} - 2230\text{H} + 51.2\text{C} \times \text{H} + 131\text{N} + 20600) \times 10^{-3} \quad (4)$$

2.4 Pyrolysis analysis

Pyrolysis of both extracted lipid and defatted microalgae samples was carried out at a fixed temperature within the temperature range 350 ℃ to 750 ℃, at temperature intervals of 100 ℃. The pyrolysis experiment, bio-oil production of samples, and subsequent analysis of the products were performed by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) [30]. Such a method is widely used due to its advantages of less sample consumption, high analysis speed, high sensitivity, and potential of

qualitative and quantitative determination of pyrolysis products [31,32]. Briefly, pyrolysis was performed using a single-shot pyrolyzer (Frontier Labs 3030i, Japan), and composition of pyrolysis products was analyzed by GC-MS (Agilent 7890A/5975C; Agilent Technologies, USA). The GC injector temperature was set to 250℃, with a split ratio of 1:10. The flow rate of carrier gas (He) was 1.0 mL min^{-1}. Chromatographic separation was performed on a capillary column (HP-5; length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm; Agilent Technologies, USA). The GC oven temperature was initially held at 40℃ for 3 min, then increased to 200℃ at 5℃ min^{-1}, held for 5 min, then further increased from 200℃ to 250℃ at 10℃ min^{-1} and finally held at 250℃ for 5 min.

After pyrolysis, GC and GC-MS data was analysed with analysis software from Agilent MSD Productivity Chem Station (Version D03.00.552; Agilent Technologies, USA), by which the total ion current (TIC) diagram was obtained from samples pyrolyzed under different temperatures. Mass spectra from each chromatographic peak were identified by comparison with spectra in the NIST2011 mass spectral library (Version 2.0, National Institute of Science and Technology, USA). Only those compounds attaining a library match greater than 80% were accepted. All experiments were conducted in triplicate for further statistical analysis.

3. Results and discussion

3.1 Pyrolysis product contents and safeness between raw and defatted microalgae

Applying pyrolysis technologies to convert microalgal biomass into bio-oil has been widely investigated during the past decades [33]. During pyrolysis, heating with absence of oxygen, thermal degradation of this material generates multiple products with varying compositions. In the present study, the pyrolysis products from defatted microalgal residues produced chemical moieties comparable with those from raw microalgae (Table 1), including aliphatic hydrocarbons, aromatic compounds, organic acids, nitrogen-containing compounds, polycyclic aromatic hydrocarbons (PAHs), and others (such as phenols, ketones, aldehydes, alcohols, and furans).

Aliphatic hydrocarbons, aromatic hydrocarbons and fatty acids were classified as major valuable compounds (MVCs) due to their re-utilization potential and high yields. Aliphatic hydrocarbons, as natural constituents of fossil fuels, and consist, in part, alkanes and alkenes [30]. Aromatic hydrocarbons are important industrial chemicals and could be using as additives for improving fuel quality [30,34]. Fatty acids, especially long-chain fatty acids, have the potential to produce not only straight-chain hydrocarbons via the denitrification and deoxygenation reaction, but also fatty acid methyl ester (FAME) through the transesterification process [28]. Previous studies indicate that lipids could be cracked into aliphatic hydrocarbons and then, through a series of thermochemical reactions, converted to aromatics during pyrolysis [35]. Therefore, this study indicated that lipid extraction significantly reduced both pyrolysis products of aliphatic and aromatic hydrocarbons from defatted microalgae samples (Table 1). In addition, the content of fatty acids, which were also mainly produced from the pyrolysis of microalgal lipids, also decreased after lipid extraction. Although not regarded as a pollutant *per se*, the existence of fatty acids could reduce the pH of the bio-oil, which would increase the corrosive potential of the product [36].

PAHs and nitrogen compounds were regarded as harmful substances (HSs), due to their toxic properties. Three processes were mainly responsible for the production of PAHs, including 1) Diels-Alder reactions, 2) deoxygenation of oxygenated aromatic hydrocarbon compounds, and 3) pyrolysis of proteins [28]. Table 1 indicates that the relative content of PAHs in pyrolysis products of defatted microalgae residues was significantly lower, compared with the products from raw microalgae, when processed at 650 ^{o}C. This might be due to the participation of pyrolysis products in secondary reactions under high temperature. Reduction of PAH levels in the final product would contribute to the overall ecological safety of the applied process.

Table 1. Pyrolysis products and relative content at 650℃.

Groups	Raw microalgae		Defatted microalgae	
	Substances	**Content（%）**	**Substances**	**Content（%）**
Aliphatic hydrocarbons	Alkanes	4.6±0.18	Alkanes	0.3±0.01
	Alkenes	14.9±1.35	Alkenes	6.0±0.29
	Total	*19.5±1.53*	Total	*6.3±0.3*
Aromatic hydrocarbons	Benzenes	21.1±1.85	Benzenes	11.9±1.06
	Indenes	0.4±0.03	Indenes	-
	Total	*21.5±1.88*	*Total*	*11.9±1.06*
Fatty acids	*Total*	*0.8±0.05*	*Total*	-
Nitrogen-compounds	Amides	2.5±0.83	Amides	-
	Indoles	4.4±0.35	Indoles	4.9±0.27
	Pyridines	2.0±0.12	Pyridines	3.2±0.39
	-	-	Pyrroles	2.6±0.32
	-	-	Nitriles	2.2±0.15
	-	-	Quinoline	0.2±0.03
	-	-	Others	0.4±0.06
	Total	*9.0±1.3*	*Total*	*13.5±1.22*
PAHs	Naphthalenes	2.2±0.21	Naphthalenes	-
	Total	*2.2±0.21*	*Total*	-
Others	Ketones	-	Ketones	-
	Alcohols	0.6±0.05	Alcohols	0.5±0.07
	Aldehydes	-	Aldehydes	1.3±0.12
	Phenols	3.2±0.41	Phenols	3.7±0.42
	Furans	1.3±0.15	Furans	0.2±0.01
	Esters	1.4±0.16	Trichloromethane	7.6±0.83
	Total	*6.5±0.77*	*Total*	*13.3±1.45*

-: not detected.

Nevertheless, microalgal residues promoted significantly higher N-containing compounds, e.g. nitriles, quinoline (Table 1) and trichloromethane in the pyrolysis products. N-containing compounds were produced mainly through protein pyrolysis, and may lead to toxic NO_x emission [36]. After lipid extraction, microalgal protein content increased in the residues, resulting in increasing amounts of N-containing compounds in the product. Thus, further optimization of the pyrolysis process should be attempted in order to reduce such toxics in the products, for instance, by changing the lipid extraction stage, and thus control the formation of N-containing compounds. Thus, futher

optimization of the pyrolysis process should be attempted in order to reduce such toxics in the products, for instance, by changing the lipid extraction stage, and thus control the formation of N-containing compounds. Besides, a proper combination of catalytic pyrolysis process (microalgal biomass), e.g., adding zeolite as catalyst, could also significantly reduce the production of oxygeneous and nitrogenous compounds, which offers a perspective of potential improvement [37] In this study, even with these limitations, the present study has demonstrated the potential of converting microalgal residues (after lipid extraction) for bio-oil production.

3.2 The effect of temperature on composition distribution

The elemental compositions of both raw and defatted microalgae biomass showed no significant differences in hydrogen, nitrogen and sulfur (Table 2). As the carbon could reach as high as 76% of the total algal lipid [38], a 15% reduction in carbon content was generated after lipid extraction than that determined from the original microalgal biomass (Table 2). This result was consistent with previous studies that carbon content decresed significantly after lipid extracted while other elements content (hydrogen, nitrogen and sulfur) only show a slight change [39, 42]. Contents of carbon and hydrogen are decisive for the HHV of a certain material. When compared with other common materials (wood, pork, and rice), and with other types of river microalgae (*Scenedesmus* sp), the lower contents of carbon and hydrogen in raw and defatted microalgae led to significantly lower HHVs [30,40-42]. Notably, similar HHVs were observed in the samples before and after lipid extraction, which may indicate the similar energy potential of microalgal residues [43]. From these results, it is clear that further investigations into the conversion of microalgal residues to energy-related products are feasible.

Table 2. Elemental compositions of pyrolysis feedstock (wt.%).

Feedstock	Elemental analysis/%				HHV /MJkg^{-1}	Chemical analysis/%		References
	C	H	N	S		Lipid content	Protein content	
Raw microalgae	25.8	5.4	6.2	0.6	12.9	18.6	38.8	This study
Defatted microalgae	21.9	4.9	5.8	0.7	12.5	-	36.3	This study
Raw Scenedesmus sp.	43.8	5.7	8.1	0.6	-	-	50.6	[42]
Defatted *Scenedesmus* sp.	42.4	5.6	8.8	0.7	-	-	55	[42]
Raw *Nanofrustulum* sp.	27.45	4.23	2.90	0.96	-	12.95	12.52	[39]
Defatted *Nanofrustulum* sp.	20.19	2.93	2.93	0.76	-	-	8.70	[39]
Wood	56.4	6.2	0.1	-	16.2	-	-	[40]
Pork	43.6	8.1	9.8	1.0	19.6	-	-	[41]
Rice	44.3	7.9	1.2	0.8	18.0	-	-	[41]

Note: '-' represents 'not reported'.

To better study the influences of temperature on composition of products, pyrolysis of both samples (raw microalgae and defatted microalgae), at temperatures in the range 350-750 ℃, was conducted. For raw microalgae biomass, with increases in pyrolysis temperature from 350℃ to 750℃, (Fig.1a), the content of aliphatic hydrocarbons presented a bimodal trend, which reached an initial maximum at 450℃ (21.4%), with a second, slightly smaller, peak at 650℃ (19.5%). During pyrolysis, microalgal lipids could be cracked into hydrocarbons [43], among which, several groups are important components for fuel applications. Therefore, optimal control of the pyrolysis process, in order to increase contents of valuable compounds, is significant. The second peak of production of aliphatic hydrocarbons may due to the continuing increase in temperature, which led to secondary cracking reactions in the raw microalgae samples [30]. Meanwhile, increase of pyrolysis temperature to 750℃ significantly elevated the content of aromatic hydrocarbons (36.9%), nitrogen compounds (9.1%) and PAHs (4.1%), but however, significantly reduced the contents of fatty acid from 16.2% to non-detectable levels. Previous studies have revealed that, with lower process temperatures (under 550 ℃), lipids produce acids and alcohols through thermal reactions [44], which is consistent with the present results.

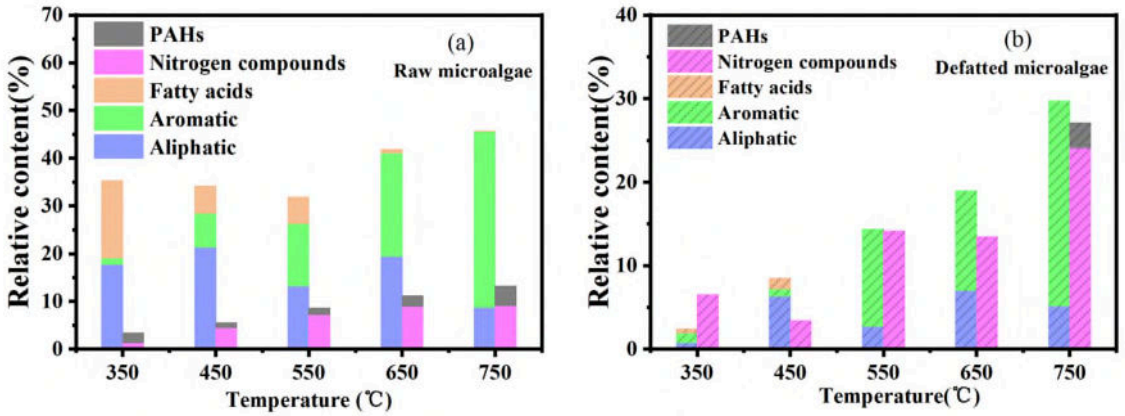

Fig. 1. The relative contents of major valuable products and harmful substances from raw microalgae (a) and defatted microalgae (b).

The determination of different pyrolysis products from defatted microalgae exhibited similar trends with temperature as those from raw microalgae (Fig. 1). A bimodal trend of aliphatic hydrocarbon concentration was also observed in the defatted samples, however, with much lower proportions (51-96% lower than those from raw microalgae). Similarly, the contents of aromatic hydrocarbons also increased with rising temperatures, but a significantly lower amount was generated by the defatted microalgae samples (1-24.5%). Both aliphatic and aromatic hydrocarbons were mainly from the pyrolysis of lipids, which had been extracted from the microalgae, defatted samples produced lower levels of aliphatic and aromatic hydrocarbons. In comparison, defatted microalgae contained a significantly higher relative protein content in the residue. During the pyrolysis process, proteins of microalgal cells could be degraded into other N-containing compounds [34], which generated significantly higher (50-366% higher) nitrogen compounds in defatted microalgae samples than from raw microalgae. As an unexpected component with corrosive properties, levels of fatty acids in pyrolysis products from defatted microalgae were significantly lower than from raw microalgae, due to the decrease of lipid contents in biomass. It could also be eliminated by increasing the process temperature above 450 ℃, which was lower than did the raw microalgae. Another group of toxic products, PAHs, was only detected at 750 ℃. During the secondary reactions of derivatives of benzene, as well as the cleavage and recombination of aromatic compounds and the condensation of free radicals, PAHs are formed [41].

Such reactions usually increase with the increasing temperature [45]. Notably, another harmful product, trichloromethane, was detected under all temperatures from defatted microalgae samples, produced by the production of chloroform through thermal reactions. With increasing reaction temperature, the relative content of trichloromethane fluctuated in the range of 5.8%-26.2%. Thus, a better extraction of lipids is necessary for reducing the occurrence of toxics or pollutants from the products, and could further help to improve the energy potential of microalgal residues.

Generally, with a change in reaction temperatures from 350℃ to 550℃, the relative content of MVCs from raw microalgae decreased slightly from 35.3% to 31.8%. At higher temperatures (650℃ and 750℃) the content of MVCs from raw microalgae increased to 41.9% and 45.7%, respectively. Moreover, the content of HSs from raw microalgae reached a maximum value of 13.2% at 750℃. Similarly, The MVCs and HSs from defatted microalgae reached a maximum content of 29.7% and 27.1% at 750℃，respectively. With comprehensive consideration, at 650 ℃, the yield of MVCs reached the second peak, while HSs only achieved half concentrations of the maximum yield (at 750 ℃). In addition, PAHs were also not detected at 650 ℃. Therefore, after due consideration involving many trade-offs, an optimal pyrolysis temperature of 650 ℃ for the processing of both types of microalgal sample, was decided upon. Futhermore, bio-oil prduction (41.9%) from defatted microalgae pyrolysis at optimal temperature (650 ℃) in this study was much higher than that in the previous study, who pyrolysis Tribonema minus at 450 ℃ with 29.82% bio-oil yield, which indicated a better performance and application for energy utilization [46].

3.3 Pyrolysis pathways of microalgae residues

Microalgae are mainly composed of carbohydrate, lipids, and proteins and, owing to the complexity of pyrolysis products, study of specific reaction pathways is critical to better explain the process. In previous studies, mechanisms and reactions during pyrolysis of microalgal biomass were proposed and discussed [44,47–49]. With reference to these

findings, and the results obtained from this study, pyrolysis reaction pathways of microalgae residues of *Desmodesmus sp*. were postulated (Fig. 2).

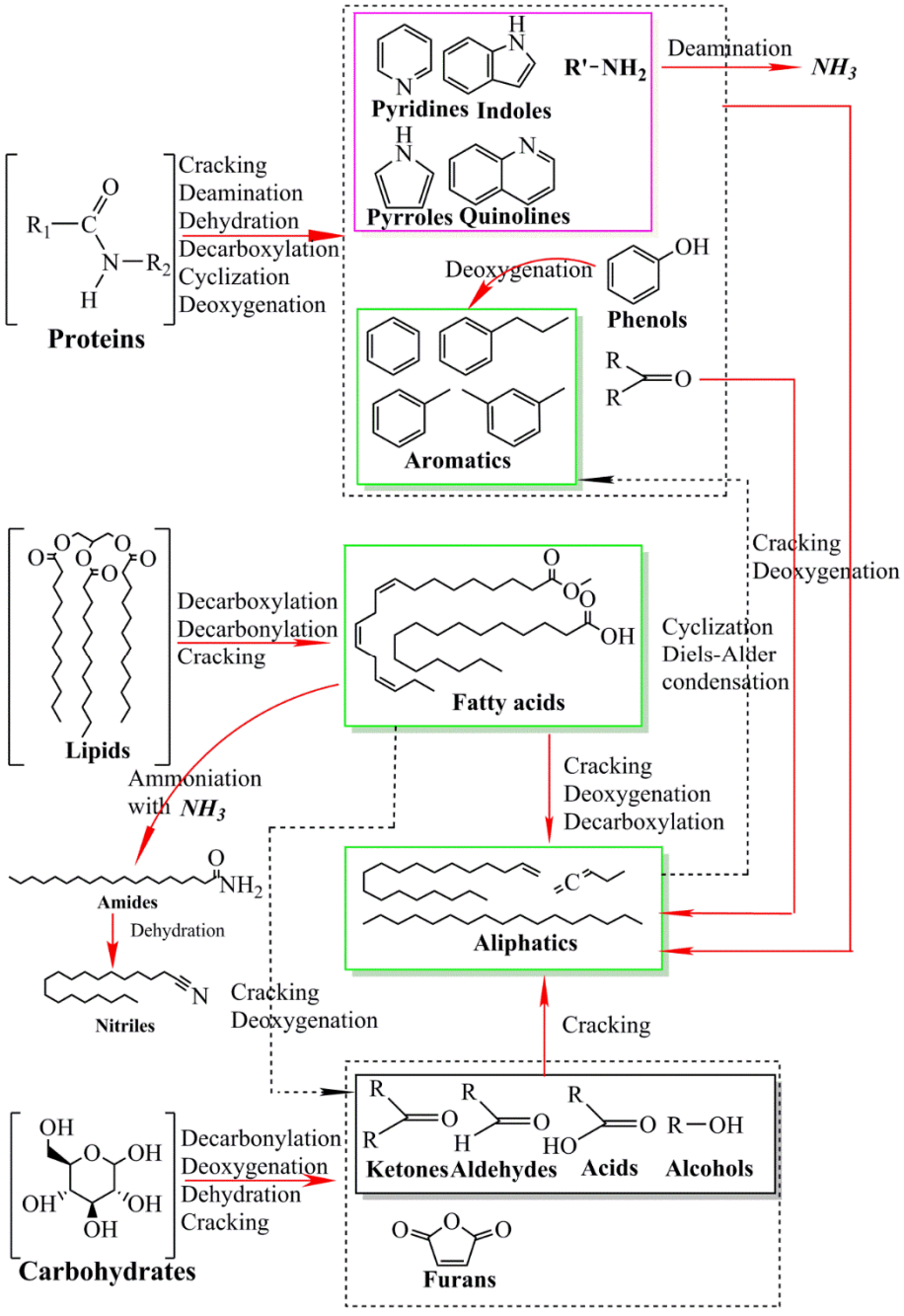

Fig. 2. Pyrolysis pathways of microalgae. Red arrow: the pathway was verified; Dashed arrow:the pathway might exist but not verified; Dashed rectangle: the products were derived from the same substance; Green rectangle: the products belong to MVCs; Pink rectangle: the products belong to HSs.

During pyrolysis of microalgae, proteins might undergo cracking, deamination, dehydration, decarboxylation, cyclization, and deoxygenation reactions that generate N-

containing compounds (such as pyridines, pyrroles, quinolines, indoles), aromatics (such as benzenes), phenols and other intermediates. The intermediates and phenols may be converted to aliphatics and aromatics by further reactions [14,47,48]. During pyrolysis, microalgal lipids may initially be converted to fatty acids through cracking or hydrolysis, and then fatty acids could be further converted to ketones, aldehydes, acids, alcohols, and some alkenes. Moreover, aromatics may further be generated through cyclization and Diels–Alder condensation reactions of alkenes [14]. Fatty acids may also react with NH_3, derived from protein pyrolysis, to produce long chain amides and nitriles [47]. From carbohydrate pyrolysis, oxygen-compounds and water may be generated. Specifically, carbohydrate could be transformed by decarbonylation, deoxygenation, dehydration, cracking, and rearrangement reactions to form furans, ketones, aldehydes, acids, and alcohols. Meanwhile, a portion of carbohydrate pyrolysis products could be further converted to aliphatics, such as alkenes.

The increase of N-containing compounds in microalgal residues after pyrolysis further proved the generation of N-containing compounds from proteins. It was found that the relative content of phenols from defatted microalgae was slightly higher than that of raw microalgae (Table 2). This also indicated that the lack of lipids may enhance the pyrolysis reaction of proteins. The relative contents of fatty acids, after lipid extraction, decreased drastically, which suggested that fatty acids were produced by lipids. At 650℃, amides were not detected in the pyrolysis products of defatted microalgae, demonstrating that fatty acids might be converted to long chain amides and nitriles. Further, the relative content of aliphatic and aromatic hydrocarbons from defatted microalgae was significantly lower than that observed from raw microalgae (Fig. 1). These phenomena demonstrated that pyrolysis of lipids could produce aliphatic hydrocarbons through fatty acids, and that these could then be converted to aromatics by cyclization and Diels-Alder condensation (Fig. 2). Moreover, after lipid extraction, the significant decrease in the relative content of aliphatic hydrocarbons was consistent with previous studies [48], where production of aliphatic hydrocarbons was mainly attributed to by the pyrolysis of

lipids. In addition, at 650℃, the relative content of aliphatic hydrocarbons from defatted microalgae was still one third that of raw microalgae, which indicated that aliphatic hydrocarbons may also have been produced as intermediates by protein pyrolysis and by part of carbohydrate pyrolysis.

Among the pyrolysis products from both raw and defatted microalgae, ketones and alcohols were at low levels, of which, ketones were only detected when the process temperature was 550℃. When microalgae were pyrolyzed after lipid extraction, the aldehyde content was significantly increased, while furans significantly decreased. Ketones, aldehydes, alcohols, and furans did not present any obvious trends with respect to relative content. This might be due to the pyrolysis of carbohydrate, which was strongly influenced by the reaction conditions, such as pyrolysis temperature and residence times [48]. The major chemical components of microalgae may also interact with each other during pyrolysis, for instance, by the Maillard reaction, which occurs between the intermediates of carbohydrate and protein during pyrolysis. Wang *et al*. used co-pyrolysis of soybean protein and glucose to reveal that some products of the Maillard reaction could further decompose to form N-heterocyclic compounds with higher contents of O and N [49]. This may explain the significant increase of nitrogenous compounds after lipid extraction. The interactions between fatty acids and proteins may also form protein-fatty acids, which was further indicated by the presence of surfactants, detergents, amides and amines in the pyrolysis products [49].

Among previous studies, pyrolysis defatted microalgae biomass of different algal species has been reported in literature, including *Nannochloropis* residues, *Chlorella* , *Chlamydomomas* and *Dunaliell tertiolecta*, which all focused on the potential energy utilization, while lacked of the pyrolysis mechanism and product pathways during the pyrolysis process which were shown in Fig.2. From this study it is clear that the effects of lipid extraction on the pyrolysis of microalgae and microalgal residues were complex. During the processing of defatted microalgae, due to the lack of lipids, protein pyrolysis

was promoted, the relative content of nitrogenous compounds increased and generated a broader range of nitrogen- containing compounds. As pyrolysis progressed further under suitable conditions, considerable amounts of aromatics and aliphatics could also be produced, indicating the energy potential of these residues. Notably, due to the focus of bio-oil, the present study concentrates on the products and pathways only regarding bio-oil, however, more products, e.g., biochar, gases, could also been generated during pyrolysis of microalgal biomass [50]. Further investigation taking biochar and gases into consideration could offer a more comprehensive insight of pyrolysis mechanism.

4. Conclusions

In the present study, effluent streams from anaerobic digestion processes were employed in the cultivation of *Desmodesmus* sp. In order to obtain microalgal biomass. The harvested microalgae and defatted residues after lipid extraction were pyrolyzed under various temperature regimes, and assessed for potential bio-fuel production. The reaction temperature significantly affected the distribution of raw and defatted microalgal pyrolysis products. The optimal temperature for pyrolysis of raw and defatted microalgae was determined to be 650℃, which produced a higher relative content of MVCs and fewer HSs. It was found that lipid extraction could significantly contribute to a reduction in the amounts of some toxic compounds produced, e.g., PAHs, but may also promote the production of nitrogen-containing compounds. Several pathways of microalgae pyrolysis were proposed, and verified. Further investigations into process optimization could enhance the feasibility of the proposed approach.

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