Catalytic Hydrothermal Liquefaction of *D. tertiolecta* for the Production of Bio-Oil over Different Acid/Base Catalysts

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In this article, two acid catalysts (ZrO_2/SO_4^{2-} and HZSM-5) and two base catalysts (MgO/MCM-41 and KtB) were used in catalytic hydrothermal liquefaction (HTL) of Dunaliella tertiolecta (D. tertiolecta) for the production of bio-oil. The results indicated that the acid/base property of the catalyst plays a crucial role in the catalytic HTL process, and the base catalyst is conducive to the improvement of conversion and bio-oil yield. When KtB was used as the catalyst, the maximum conversion and bio-oil yield was 94.84 and 49.09 wt %, respectively. The detailed compositional analysis of the bio-oil was performed using thermogravimetric analysis, elemental analysis, FT-IR, and GC-MS. The compositional analysis results showed that the introduction of catalyst is beneficial for reducing the fixed carbon content in the bio-oil, and the structure of catalyst influences on the bio-oil composition and boiling point distribution. Based on our results and previous studies, the probable catalytic HTL microalgae model over various catalysts can be described that the main chemical reactions include ketonization, decarboxylic, dehydration, ammonolysis, and so forth. with HZSM-5 and MgO/MCM-41 as the catalyst; the cyclodimerization, decomposition, Maillard reaction, and ketonization are the main reactions with ZrO_2/SO_4^{2-} as the catalyst; the dehydration, ammonolysis, Maillard reaction, and ketonization can occur with KtB as the catalyst. Therefore, a plausible reaction mechanism of the main chemical component in D. tertiolecta is proposed. *AIChE J*, 61: 1118–1128, 2015
Keywords: catalytic hydrothermal liquefaction, bio-oil, acid/base catalyst, mechanism, D. tertiolecta

Introduction

In the last century, the world has been heavily relying on fossil fuels for energy and chemical production.[1] With the eventual depletion of fossil fuels, the need for the development of sustainable renewable energy has become a global theme aimed at settling the energy crisis and environmental pollution. Due to the contradictions between the nonrenewable fossil resources and ever-increasing demand for energy the switch from fossil to renewable feedstock for the production of chemicals and fuels has recently attracted significant attention.[2] Bio-oil production from biomass, which regards as the only renewable feedstock for the production of liquid fuel, is considered to be one of the most sustainable alternatives to petroleum because of viable means for environmental and economic sustainability.[3,4] Among biomass resources, microalgae are viewed as next generation fuel feedstocks due to their superior photosynthetic efficiencies, growth rate, and area-specific yield and higher carbon capturing capabilities compared to terrestrial plants.[5,6] Microalgae, which can be used in environmental applications such as greenhouse gas biomitigation, wastewater treatment processes, and biosorption of heavy metals, are gaining attention as an alternative source of the production of bio-oils and meet the government policies.[7,8]

Microalgae are made up of three major biochemical components, namely carbohydrates, lipids, and proteins, which are an important distinguishing feature of microalgae compared to others terrestrial biomass.[9,10] The original concept for converting algal biomass into bio-oils involved lipid extraction for the production of biodiesel via transesterification. In contrast to this route in which only the lipids in the algae are used for fuel, thermochemical routes involve conversion of the entire algal organism, including the proteins and carbohydrates, into fuel oil.[11–14] Among the thermochemical conversion, hydrothermal liquefaction (HTL), performing the biomass conversion reaction used sub/supercritical water as the reaction medium avoids the necessity to dry the feedstock, is one of the most promising methods for the conversion of microalgae to bio-oil.[6,15] HTL has been studied by many researchers for microalgal liquefaction.[16–21] There is, however, one challenge still ahead-the poor bio-oil quality including a higher O and N content, lower higher heating value (HHV), and poor stability, these properties prevents its direct use as fuel for transportation applications.

To this end, an appropriate catalyst can be recommended to solve this problem. Therefore, catalytic HTL, which can

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improve the bio-oil quality and yield, has been considered as one of the most promising method in the production of bio-oil from microalgae. The majority of studies that carried out catalytic HTL used homogenous catalysts including Na_2CO_3, CH_3COOH, KOH, HCOOH, $[Ca_3(PO_4)_2]$, and so forth.[12,22–26] Both proteins and lipids in the microalgae were converted to bio-oil more efficiently without catalysts, whereas carbohydrates were better processed using Na_2CO_3.[22] In some case, the addition of the catalyst may not increase the bio-oil yield but may alter the product distribution.[25] In addition, the use of organic acids can improve the flow properties and lower the boiling point of the bio-oil.[24]

Only a few articles have used heterogeneous catalysts, such as molecular sieve, modified molecular sieves, transition metal oxide, and supported metal.[26–28] The most extensive reports on the influence of heterogeneous catalysis on HTL were published by Duan and Savage,[27] who produced bio-oils from microalgae (*Nannochloropsis sp*.) in the presence of six heterogeneous catalysts [Pd/C, Pt/C, Ru/C, Ni/SiO_2-Al_2O_3, sulfided CoMo/γ-Al_2O_3, and zeolite (aluminum silicate)]. The bio-oil yield in the absence of catalysts was 35.0 wt %, but increased to 57.0 wt % when the Pd/C catalyst was used in the absence of hydrogen. The treated bio-oil catalyzed by Pd/C, Pt/C, Ru/C, and sulfided Co-Mo/γ-Al_2O_3 has reduced the O/C ratio, meanwhile exhibited an apparently lower viscosity and lighter color than the noncatalyzed or zeolitecatalyzed samples. NiO was also used to assist in the HTL of both single (*Spirulina*) and mixed algae.[28]

In general, the HTL of microalgae, which is based on the chemical reaction of the biomacromelocules in microalgal cell, starts from the hydrolysis reaction in HTL process.[13] Previous studies have indicated that the product from HTL of microalgae is largely dependent on microalgal species, reaction temperature, holding time, and catalyst type.[17,25] The acid/base properties of catalyst seems to play a crucial role in the biochemical breakdown of microalgae because the hydrolysis reaction is directly related with acid/base catalyst, however, the relationship between the catalyst with acid/base properties and HTL of microalgae still is not clear yet.

Previous published works on production of algal bio-oil via catalytic HTL has considered as both the type of catalyst and catalytic performance.[12, 22–28] The interest of this study includes understanding the influence of catalyst acid/base with properties on the conversion and the bio-oil yields as well as the product distribution. In this article, two solid acid catalysts (ZrO_2/SO_4^{2-} and HZSM-5) and two solid base catalysts (MgO/MCM-41 and KtB) were used in catalytic HTL of *D. tertiolecta* for the production of bio-oil with an emphasis on the effect of the catalyst with acid/base properties on the catalytic performance, and the blank experiment was used for comparison. The detailed chemical compositional analysis of the bio-oil was performed using a thermogravimetric analysis (TGA), elemental analysis (EA), fourier transform infrared spectrum (FT-IR), and gas chromatography-mass spectrometry (GC-MS). In addition, a plausible catalytic reaction mechanism of the main chemical component in *D. tertiolecta* is proposed based on the experimental results and the references.

Experimental

Materials

Low-lipid microalgae *D. tertiolecta* was obtained from Xi'an Victory Biochemical Co. (Xi'an, China). The samples were prepared through pulverization in a mortar to < 75 μm and dried at 105°C for 12 h prior to use. The proximate and ultimate analysis results of the sample and its structural compositions are given in Table 1. The Analysis methods were reported in our previous work.[16,17]

Potassium *tert*-butoxide (KtB) was purchased from Sun Chemical Technologies Co. (Shanghai, China). HZSM-5 (Si/Al = 250) molecular sieve was obtained from Tianjin Chemist Technology Development Co. (Tianjin, China). MgO/MCM-41 catalyst was synthesized according to the method described in the literature,[29] the experimental procedure as follows: A mixture containing 8.0 g of calcined MCM-41, 12.8 g of $Mg(NO_3)_2{\cdot}6H_2O$ and 100 mL of deionized water were mixed and stirred at room temperature for 4 h. The sample was dried at 80°C for 12 h, calcined at 550°C for 4 h, and labeled as MgO-MCM-41. ZrO_2/SO_4^{2-} catalyst was prepared based on the method presented in the previous paper,[30] the experimental procedure as follows: 3.5 g $Zr(NO_3)_4{\cdot}5H_2O$ was dissolved in 150 mL deionized water, kept stirring while slowly dropping 10% of the ammonia, simultaneous determination of acidic solution changes to pH = 9, then stopped stirring, standing, precipitation, aging 24 h, filtered, and washed with distilled water 3–4 times, then the precipitate was dried at 110°C for 14 h, with 1 mol L^{-1} dilute H_2SO_4 impregnate 10 h, filtration, light dry, calcined at 550°C for 4 h and obtained the ZrO_2/SO_4^{2-} catalyst.

Experimental procedure

The liquefaction procedure mentioned in our previous reports was performed in a stainless autoclave with 50 mL capacity.[16,17] The autoclave was heated with an external electrical furnace, and the temperature was measured with a thermocouple and controlled to $\pm$1°C. In a typical run, *D. tertiolecta* (approximately 4.0 g), catalyst (0.4 g, the catalyst dosage is nearly equal to 10 wt % of algae), and water were fed into the reactor, then sealed, and heated up to the desired reaction temperature (360°C), and the temperature was kept for 30 min. When the reaction ended, the electric furnace was removed, and the autoclave was cooled down to room temperature. The blank experiment, which designated as "blank" was used for comparison, is carried out in absence of any catalysts.

Table 1. Analysis Results of *D. tertiolecta*

Industrial analysis/wt %		Chemical composition analysis/wt %		Elemental analysis/wt %	
Moisture W_{ar}	1.60	Lipids	5.18	C	43.31
Volatiles V_{ar}	64.70	Proteins	30.63	H	5.96
Ash A_{ar}	16.50	Carbohydrates	52.31	O[a]	29.90
Fixed carbon C_{Far}	17.20	Others[a]	11.88	N	4.33
				Empirical formula	$CH_{1.65}O_{0.52}N_{0.09}$
				HHV (MJ kg^{-1})	17.81

[a]Calculated by difference.

The separation of catalytic liquefaction products is depicted in Figure 1. The products included the gaseous, organic phase, and water-soluble phases, as well as the solid residue (SR). The gaseous products were selected after the autoclave had cooled down, and were direct determined via GC-MS analysis. The liquid phase fraction and the autoclave wall were washed with dichloromethane thrice, and the contents were separated through dispersion. The dichloromethane solvent was removed in a rotary evaporator at 40°C under reduced pressure, and the liquid fraction remained was called "bio-oil." The water-insoluble fraction remaining on the filter paper was dried at 105°C for more than 24 h, then weighed, and designated as the "SR." Since the organic compounds in the aqueous phase cannot be analyzed by GC-MS method directly before appropriate pretreatment. Therefore, two pretreatment methods, namely solid-phase microextraction (SPME) and dried aqueous extracts (DAE) were used for the pretreatment of the aqueous phase. After that, the organic products obtained from pretreatment (SPME and DAE) of aqueous phase were direct determined via GC-MS analysis.

The TGA experiments were carried out in a TA Instruments SDT Q600 thermogravimetric analyzer. In each experiment, alumina crucible was used to hold 10 mg of sample uniformly at the bottom and the crucible was displaced at the same position of the beam platform of the thermal analyzer. Subsequently, the sample was heated from room temperature to 800°C at the heating rate of 10°C min^{-1} under a high purity nitrogen flow of 100 mL min^{-1}. Each experiment was repeated at least twice to ensure the repeatability. Every bio-oil sample was analyzed by three runs repeatedly at the same conditions, and taken the average as the final results.

The elemental composition was performed on a CE440 elemental analyzer. The HHV was calculated according to the Dulong formula[31]

$$\text{HHV } (\text{MJ}\cdot\text{kg}^{-1}) = 0.3383\text{C} + 1.422(\text{H-O}/8) \quad (1)$$

where C, H, and O are the weight percentage of carbon, hydrogen, and oxygen in the samples, respectively.

FT-IR analysis was performed on a Spectrum GX series FT-IR spectrometer to determine the functional groups. GC-MS analysis was carried out on a Trace DSQ GC-MS system with an AB-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness), Helium was used as carrier gas with a flow rate of 1 mL min^{-1}. The column temperature was programmed from 70 to 300°C at a rate of 10°C min^{-1} after an initial 2-min isothermal period, and then it was kept at the final temperature for 10 min. The inlet temperature was set to 300°C, and the split ratio was 1:50. The mass spectrometer was set to an ionizing voltage of 70 eV with a mass range from 35 to 650 amu.

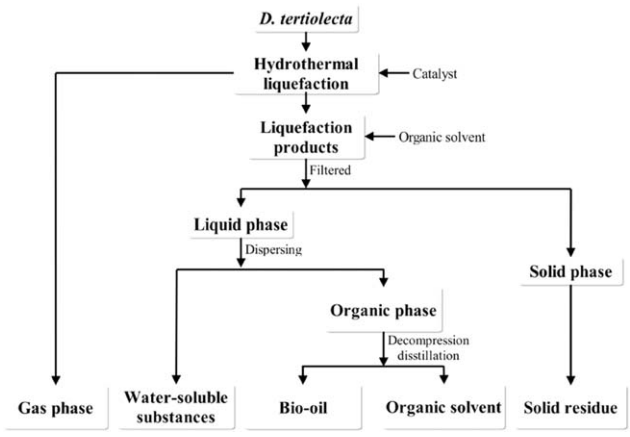

Figure 1. Procedure for the separation of reaction products.

NH_3-TPD and CO_2-TPD were carried out with a TP-5076 multifunctional automatic adsorption instrument (Xianquan Industry and Trade Development Co., Tianjin, China) to determine the acid site density and basic site density of the used catalysts. NH_3-TPD measurements of ZrO_2/SO_4^{2-} and HZSM-5 were carried out to determine the amount and relative strength of acid sites. Typically 0.1 g of a sample was used. The sample was pretreated at 500°C in a flow of He (30 mL min^{-1}) for 1 h and adsorbed a flow of NH_3 (15 mL min^{-1}) at 100°C for 30 min, and then purged with He until the physically adsorbed NH_3 was removed. The sample was then heated under a flow of He (30 mL min^{-1}) from 100 to 800°C with a ramp of 5°C min^{-1}. The signals of NH_3-TPD were recorded with an online thermal conducted detector. CO_2-TPD measurements of MgO-MCM-41 and KtB were carried out according to the NH_3-TPD procedure.

The bio-oil yield and the *D. tertiolecta* conversion, were expressed in %w/w, and calculated based on the dry organic matter as follows

$$\text{Bio-oil yield } = \omega_1/\omega_0 \times 100\% \quad (2)$$

$$D.\ tertiolecta \text{ conversion} = 1 - \omega_2/\omega_0 \times 100\% \quad (3)$$

where ω_o (g), ω_1 (g), and ω_2 (g) were defined as the mass of *D. tertiolecta*, the bio-oil, and the SR, respectively.

Energy recovery of bio-oil was defined as the ratio of HHV of bio-oil against that of the microalgal feekstock, which can be calculated as follows[25]

$$\text{Energy recovery of bio-oil (wt \%)} = \frac{E_1 \times \omega_1}{E_0 \times \omega_0} \times 100\% \quad (4)$$

hereinto, ω_0 (g) and ω_1 (g) were defined as the mass of *D. tertiolecta* and bio-oil; E_0 (MJ kg^{-1}) and E_1 (MJ kg^{-1}) as the HHV of *D. tertiolecta* and bio-oil, respectively.

Results and Discussions

Effect of acid/base property of catalyst on the catalytic HTL

As mentioned above, the major biochemical components of *D. tertiolecta* are proteins, carbohydrates and lipids.[9,10] Different biochemical components could show different transformation process in the catalytic HTL process.[25] To this end, ZrO_2/SO_4^{2-}, HZSM-5, MgO/MCM-41, and KtB were used as the catalysts in HTL of *D. tertiolecta*. The acid/base densities of the used catalyst were measured by NH_3-TPD and CO_2-TPD, the analysis results are presented in Figure 2. The acid strength trend of the acid catalyst is $ZrO_2/SO_4^{2-} >$ HZSM-5, which is similar with the publication paper.[29] As for solid base catalyst, the literature reference had demonstrated that MgO-MCM-41 catalyst exhibited weak base.[30] In spite there is a strong base site in MgO-MCM-41 catalyst, however, KtB can hydrolysis to form homogenous KOH, which can enhance the alkalinity in hydrothermal condition, the base strength trend as follows: KtB > MgO-MCM-41 in the sub/supercritical water.[32]

The bio-oil yields and conversions are plotted against the type of catalyst as shown in Figure 3. As can be seen from

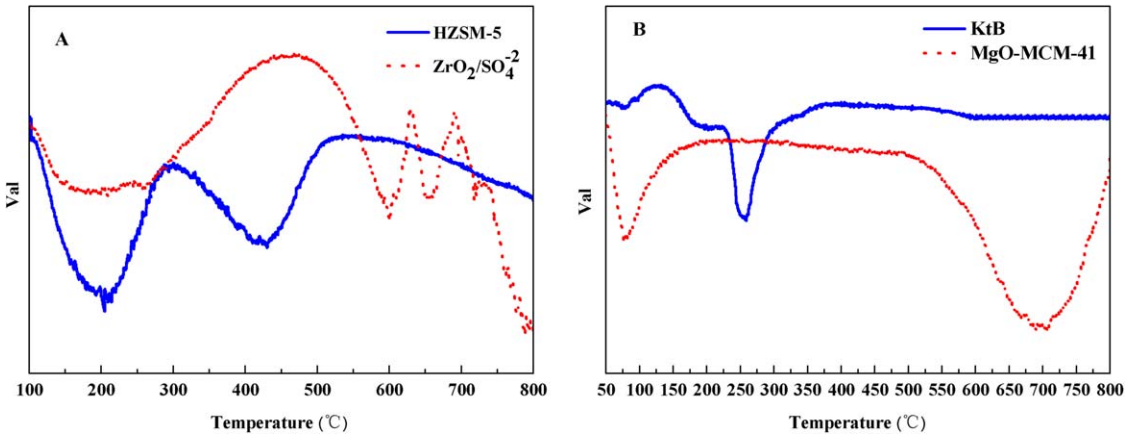

Figure 2. NH_3-TPD profiles (A) of ZrO_2/SO_4^{2-} and HZSM-5 and CO_2-TPD profiles (B) of KtB and MgO-MCM-41.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3, the acid catalysts showed the lower conversion and bio-oil yield compared to the base catalysts and noncatalytic HTL results. In other words, according to the conversion and bio-oil yield, the catalytic performance of the used catalysts follows the trend of KtB > MgO/MCM-41 > Blank > ZrO_2/SO_4^{2-} > HZSM-5.

The reasons for these results may be related to the main biochemical components in *D. tertiolecta*. As for noncatalytic HTL, the conversion of three components in *D. tertiolecta* (proteins, carbohydrates and lipids) decreases in the sequence of lipids > proteins > carbohydrates.[25] Lipid and protein can be converted effectively in the condition of either acid or base circumstance, even in the absence of catalyst, the conversion of lipid and protein can also occur when sub/supercritical water was used as the reaction medium.[22] However, the base circumstance is more conducive to the transformation of carbohydrates in the HTL.[25] In general, when acid catalyst ZrO_2/SO_4^{2-} and HZSM-5 were used, the acid environment reaction system improved the conversion of lipids and proteins, but the carbohydrates cannot be converted efficiently. In addition, the acid environment may lead to cracking reaction, and more organic compounds decompose into small molecules transferring to water phase, therefore, the conversions and bio-oil yields are less than that of obtained in the blank experiment.

Conversely, when base catalyst MgO/MCM-41 and KtB were used, the base reaction system can not only be conducive to the conversion of carbohydrates fraction in microalgae, but also accelerate the condensation and polymerization reaction, thus the bio-oil yields was greatly elevated. Especially for KtB catalyst, the microalgal conversion and the bio-oil yield were up to 94.84 and 49.09 wt %, respectively. Besides, the high conversion and bio-oil yield may be related to *tert*-butyl oxygen radicals produced from the hydrolysis of KtB.[32] *Tert*-butyl oxygen radicals, which is a highly reactive free radicals with high alkalinity, can effectively cause the chain reaction, for example, condensation, and then some organic small molecule compounds can be converted to hydrophobic macromolecules and enter into the oil phase.[25] In addition, the use of commercial KtB has many advantages[32]: free from solvation, stability in storage, and its structural lack of hydrogen atoms for self-deprotonation.

Otherwise, another hydrolysis product of KtB is KOH, which is the common homogeneous catalyst in the HTL of microalgae due to its advantage of mass and heat transferring.[12] To confirm whether the excellent catalysis effect of KtB is related to the homogeneous catalysis process, KOH was used as the catalyst under the same conditions for comparison, the experimental results is shown in Table 2. As can be seen from the comparison of experimental results between KtB and KOH, the conversion and bio-oil yield obtained with KOH as the catalyst is lower than KtB does, but higher than that of other catalyst and blank experimental results. Therefore, the acid/base properties of catalyst plays a crucial role in the process of catalytic HTL of microalgae, and strong basic catalysts (KOH) with amorphous structures give the best conversion and selectivity to bio-oil. In addition, the property of mass and heat transferring is another important factor influencing on the HTL effect. Additionally, Na_2CO_3 is the common catalyst used in the catalytic HTL of microalgae.[12, 22–25] However, the catalytic performance of KOH is

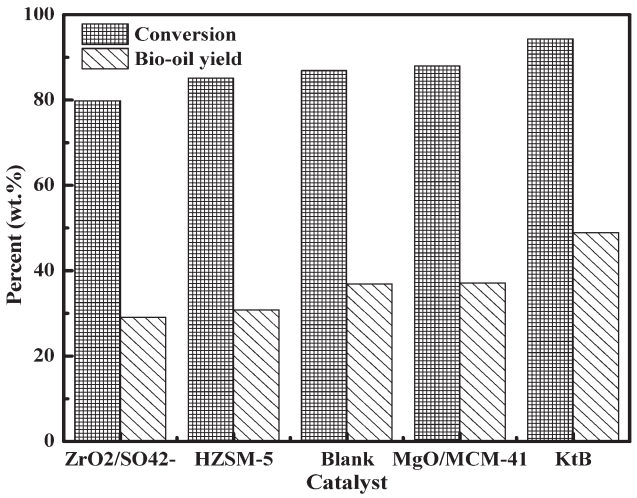

Figure 3. Effect of acid/base property of catalyst on the conversion and bio-oil yield.

Table 2. Comparison of KOH and KtB on the Conversion and Bio-Oil Yield

Catalysts type	Conversion (wt %)	Bio-oil yield (wt %)
KOH	90.09	41.78
KtB	94.84	49.09

superior to Na_2CO_3,[12] therefore, KOH was selected as the only catalyst for comparison.

TG analysis of catalytic HTL product

It is clear that the bio-oil obtained from catalytic HTL of microalgae is a very complex mixture, and the component in the bio-oil is widely distributed.[13,17,25] TGA applied in simulated distillation is regarded as a miniature "distillation." Notwithstanding some thermal degradation likely occur, TGA provides an estimate of the boiling range of bio-oil.[33,34] In this article, TGA of bio-oil obtained from catalytic HTL of *D. tertiolecta* over various catalysts was carried out, and the TGA and DTG results are presented in Figure 4. The TGA curves indicated that the introduction of catalysts can reduces the boiling point range of the bio-oil compared to blank results. Heating of bio-oil under an inert atmosphere to 800°C resulted in a weight loss of about 90 wt % in the present of catalysts. In contrast, when the catalysts is absent in the reaction system, the weight losses of bio-oil is lower 80 wt % (Figure 4A), this results may be related to the fixed carbon remained in the bio-oil. That is to say that the adding of catalyst can help to decline the fixed carbon content in the bio-oil. As can be seen from Figure 4A, when HZSM-5 and MgO/MCM-41 were used as the catalyst, the weight loss curves of bio-oil almost overlap and the initial temperature of weight loss is also similar. These results are owed to the similar bio-oil products, and the reason for this similarity could be a result of transport limitations restricting the conversion to catalytic sites that are external to the pores.

DTA curves of bio-oils are presented in Figure 4B. It is clear that the bio-oil can be roughly divided into three regions: The first region from room temperature to 150°C belongs to light component; the second region from 150 to 350°C belongs to the middle component, which is characterized by a major weight loss, corresponding to the main weight loss of bio-oil. There is a strong peak in the rate of weight loss curve, with a peak temperature at 250°C, at which the rate of weight loss attains the maximum. The third region from 350°C to the final temperature (800°C) belongs to the heavy component. The light component in the bio-oil obtained from HZSM-5 catalysis is in the majority at 100°C with the peak of weight loss; however, the heavy component is very few. In addition, the DTG curve of the bio-oil over MgO/MCM-41 is similar to the results obtained from HZSM-5 catalysis.

It is worth noting that the blank value and KtB catalyst showed few light components content in the bio-oil with relatively more amount in heavy component. This phenomenon can be explained from the following several aspects[13]: on the one hand, for the blank, the major components in the microalgae were converted into bio-oil through the hydrolysis process in the absence of any catalysts, and sub/supercritical water plays an important role in the HTL process, thus the liquefaction process is not completely, there are a large number of macromolecular substances existed in the bio-oil so that the quantity of heavy component is large. On the other hand, when KtB was used as the catalyst, the effective conversion of microalgal carbohydrates led to the significant increase of bio-oil yield, however, the strong base condition is advantageous to the condensation reaction resulting in the enhancement of heavy component in the bio-oil.

Figure 5 displays the boiling point distribution of the bio-oil obtained from catalytic HTL. The bio-oil obtained from HZSM-5 and MgO/MCM-41 represented the similar boiling point distribution, and the lower boiling point implies having a lower molecular weight compared to the blank one. Although the boiling point distribution graph does not provide exact information for each chemical in the bio-oil,[35] the HTL of microalgae is likely to decompose the microalgal biomass into smaller molecules. The mass portions of diesel in the bio-oil with the catalyst of HZSM-5 and MgO/MCM-41 were both 50 wt %, and that of ZrO_2/SO_4^{2-} and KtB as well as blank is close to 60 wt %. Therefore, the catalysts (HZSM-5 and MgO/MCM-41) gave the best in the boiling point distribution and the high quality to bio-oil can be obtained with the proper boiling point range via catalytic HTL.

FT-IR analysis of catalytic HTL product

FT-IR analysis of the bio-oils and SR obtained from catalytic HTL allowed for a more comprehensive comparison of organic compounds through the functional group characteristics with spectral band assignments and interpretation based on previous studies.[16,17,27,36,37] The possible structures and functional groups of the bio-oil and SR are as shown in Figure 6. Compared with the IR adsorption profiles of *D.*

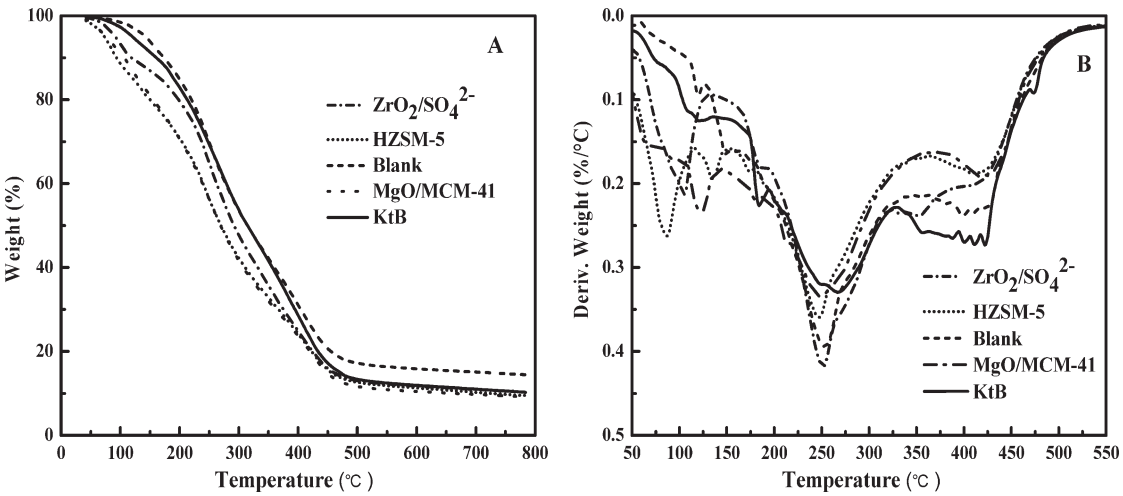

Figure 4. TGA (A) and DTG (B) results of the bio-oil obtained from catalytic HTL on various catalysts.

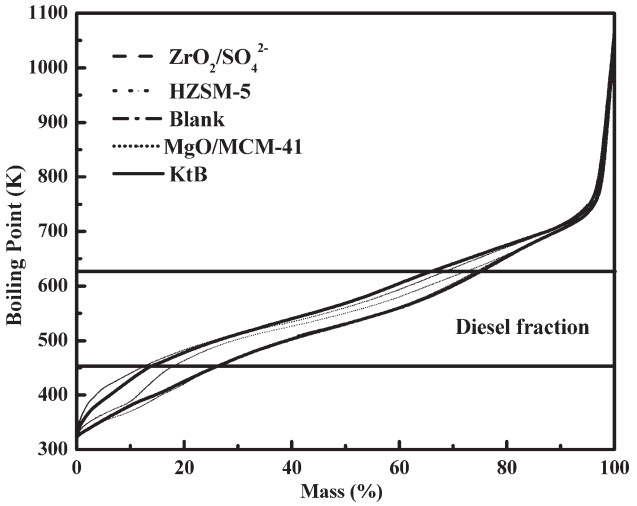

Figure 5. Boiling point distribution of the bio-oil obtained from catalytic HTL on various catalysts.

tertiolecta in the literature,[17] the absorption between 3100 and 3600 cm^{-1} weakened obviously in the bio-oil (Figure 6A). This finding suggested that the protein (amino acid) and/or polysaccharide compounds were converted into other molecules in the HTL, resulting in the concentrations of the amino-containing and/or hydroxyl-containing compounds also decrease. The absorptions between 2800 and 2960 cm^{-1} could be attributed to the symmetrical and asymmetrical C—H stretching vibrations of the methyl and methylene groups, respectively.[16] The presence of both O—H and C=O stretching (absorption around 1700 cm^{-1}) vibrations may also indicate the existence of carboxylic acids and their derivatives. The band at 1379 cm^{-1} was attributed to C—H bending mode.[16,17] Meanwhile, the bands between 700 and 900 cm^{-1} indicated the presence of single, polycyclic, and substituted aromatic groups. It is worth noting that the FT-IR spectra derived from the five bio-oils are very similar due to the similarity of the compounds structure. However, there are still some subtle differences in the wave number of 1700 cm^{-1}. When HZSM-5 was used as the catalyst, the absorption of 1700 cm^{-1} showed a slight shift to the direction of low wave number, meanwhile, the peak strength is weaker than the others. This indicated that it is given priority to with ketone compounds in the bio-oil.

When considering the FT-IR spectra of the SR obtained from catalytic HTL on various catalysts (Figure 6B), the bands of the ether groups (1000–1200 cm^{-1}) are relatively stronger than those in bio-oil, which suggests that the ether groups can be improved after dehydration and condensation reactions.[17] There were no characteristic absorption peaks from 1400 to 4000 cm^{-1}; thus, most of the proteins, carbohydrates, and/or lipids had been transferred into other chemical products in the HTL of *D. tertiolecta* process.[17] Interestingly, there are nearly no significant absorption peaks at 400–4000 cm^{-1} using ZrO_2/SO_4^{2-} as the catalyst as well as blank. This shows that the organic compounds in raw materials were converted more thoroughly in the two processes, the majority of H, O, and N are transformed effectively, and the SRs are mainly in the form of fixed carbon. In addition, the characteristic absorption peaks located at lower than 800 cm^{-1} belongs to the presence of benzene when HZSM-5 and MgO/MCM-41 as the catalyst, which illustrated that the catalytic process is conducive to the formation of the benzene.

EA and HHV of catalytic HTL product

The physical and chemical properties of bio-oil, which directly affect its application and efficiency, are very important to estimate the treatment technology and the selection of the process equipment. EA can provide the information of element composition and higher heating values (HHVs) in the bio-oil. It is of great significance to study the bio-oil quality.[16] Table 3 summarizes the results of EA, HHVs, and energy recover of bio-oils obtained from catalytic HTL of *D. tertiolecta*.

The C and H content of the bio-oil were much higher than that of *D. tertiolecta*, whereas the O content in the bio-oil was reduced greatly. Of course, the increased C and H levels and reduced O content lead to the having a higher energy density of bio-oil. Since the quality of bio-oils is significantly affected by its O/C ratios, it seems that the used acid and base catalyst were not advantageous in terms of bio-oil

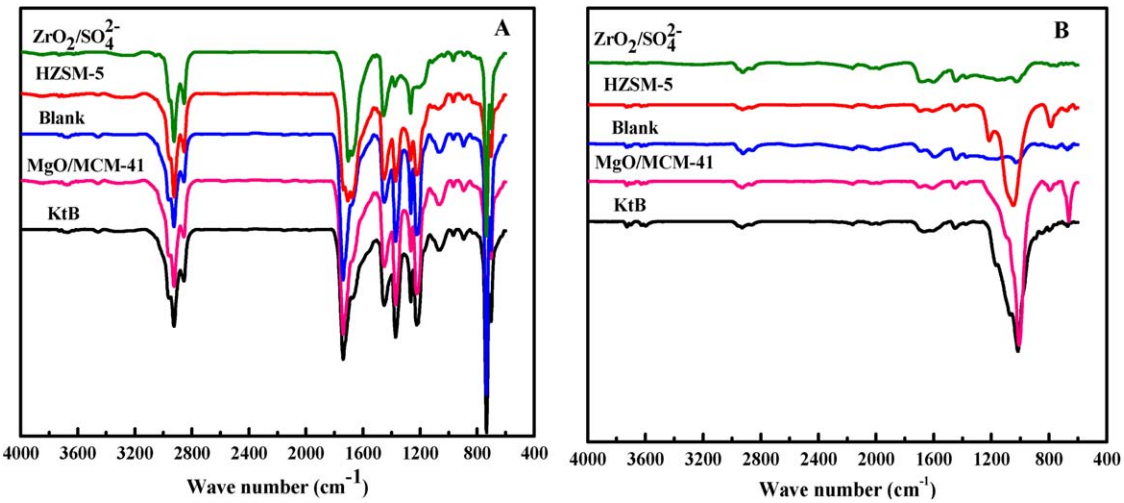

Figure 6. FT-IR results of the bio-oil (A) and SR (B) obtained from catalytic HTL of *D. tertiolecta* on various catalysts.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. Elemental Analysis, HHV, and Energy Recovery of the Bio-Oil Obtained from Catalytic HTL on Various Catalysts

Properties	ZrO_2/SO_4^{2-}	HZSM-5	Blank	MgO/MCM-41	KtB
C (wt %)	70.30	72.49	75.06	70.66	70.17
H (wt %)	8.78	8.28	8.86	8.59	8.22
O[a] (wt %)	17.03	14.78	11.68	16.57	17.22
N (wt %)	3.89	4.46	4.41	4.19	4.40
H/C	1.50	1.37	1.42	1.46	1.41
O/C	0.18	0.15	0.12	0.18	0.18
Empirical formula	$CH_{1.50}O_{0.18}N_{0.05}$	$CH_{1.37}O_{0.15}N_{0.05}$	$CH_{1.42}O_{0.12}N_{0.05}$	$CH_{1.46}O_{0.18}N_{0.05}$	$CH_{1.41}O_{0.18}N_{0.05}$
HHV (MJ kg^{-1})	33.24	33.67	35.92	33.17	32.36
Energy recovery (wt %)	54.25	58.28	74.44	69.06	88.83

[a]Calculated by difference.

quality: the corresponding O/C ratios were located in the undesired area compared to the blank results. The composition analysis is suggested to further determine product distribution in the bio-oil. The energy recovery equation of the bio-oil uses the amount of bio-oil produced and takes its HHV into account. It does not compensate for any processing energy used in the liquefaction reaction.[25] The KtB as the catalyst has the best energy recovery rate (88.83%) due to the higher bio-oil yields while for the MgO/MCM-41 the energy recovery is little lower (69.06%) due to the lower bio-oil yield. In addition, the energy density of bio-oil was greatly raised after HTL, the highest HHV of blank experiment was 35.92 MJ kg^{-1}, followed by HZSM-5 as the catalyst, nearly 33.67 MJ kg^{-1}, the minimum value of HHV is 32.36 MJ kg^{-1} using KtB as the catalyst. Overall, the bio-oil is high in oxygen content, so it is not stable. That is to say that the bio-oil is difficult to store under normal temperature due to the probable oxidation reaction. Compared to the blank result, the catalyst plays an important role to improve the quality of bio-oil due to the decrease of the N content in the bio-oil.

Table 4 lists the results of EA and HHVs of SR obtained from catalytic HTL of *D. tertiolecta*. As can be seen from Table 4, the HHV of SR obtained from MgO/MCM-41 catalytic HTL gains the minimum value (8.11 MJ kg^{-1}), and ZrO_2/SO_4^{2-} and HZSM-5 catalytic HTL represented the lower HHV (13.56 and 12.38 MJ kg^{-1} respectively) than *D. tertiolecta*, while Blank and KtB catalytic HTL display a higher HHV (25.22 and 19.99 MJ kg^{-1}, respectively) than *D. tertiolecta*. This result may be due to the high C content in SR, and is in accordance with the results of the FT-IR. Compared to the blank, the adding of the catalyst is conducive to the transformation of the protein due to the decrease of the N content in SR.

C-balance is a crucial topic on the feedstock conversion during HTL microalgae. Similar study on the HTL microalgae had reported the C-balance.[13] The carbon content is defined as the ratio of carbon in the HTL product to the carbon in the original microalgal feedstock. The C content in gaseous, bio-oil, aqueous phase, and SR was calculated according to the reported the Ref. [13

$$\text{Carbon content of product}\,(\%) = \frac{\text{C\% of the product} \times \text{weight of the product}}{\text{weight of the carbon in dry matter of microalgae}} \times 100 \quad (5)$$

To confirm the C-balance during HTL microalgae, the carbon content of both feedstock and HTL products were analyzed using a CHN analyzer. The carbon content of gaseous phase was estimated based on the GC-MS results. It is worth noting that the carbon content for the aqueous product was calculated by difference once the other three components were determined. The carbon contents in different phases after HTL, including bio-oil phase, solid phase, gas phase, and aqueous phase (aqueous product), are presented in Table 5. As can be seen from Table 5, the carbon contents of different phases after HTL in absence of catalyst are similar with the reported paper.[13]

GC-MS analysis of catalytic HTL products

The GC-MS system was used in this work to identify the main components of the five microalgal bio-oils obtain from catalytic HTL or blank HTL. The identification of GC-MS peaks was based, in most cases, on a comparison with the spectra of the NIST 98 spectrum library. The components of bio-oils were very complex and presented different distributions.[17] Detailed components and percentages of low-boiling compounds ($bp < 350°C$) in the bio-oil revealed distinct chemical class distributions for the major compounds comprising >1% of the total ion chromatogram (TIC) are detailed in Table 6. The relative percent area for each compound identified (defined by the percentage of the chromatographic area of the compound out of the total area). It

Table 4. Elemental Analysis and HHV of the SR Obtained from Catalytic HTL on Various Catalysts

Properties	ZrO_2/SO_4^{2-}	HZSM-5	Blank	MgO/MCM-41	KtB
C (wt %)	45.93	44.11	64.80	37.34	55.52
H (wt %)	4.38	4.24	5.45	3.85	5.24
O[a] (wt %)	46.14	48.26	24.99	56.28	35.06
N (wt %)	3.57	3.40	4.77	2.54	4.19
H/C	1.14	1.15	1.01	1.24	1.13
O/C	0.75	0.82	0.29	1.13	0.48
Empirical formula	$CH_{1.14}O_{0.75}N_{0.07}$	$CH_{1.15}O_{0.82}N_{0.07}$	$CH_{1.01}O_{0.29}N_{0.06}$	$CH_{1.24}O_{1.13}N_{0.06}$	$CH_{1.13}O_{0.48}N_{0.06}$
HHV (MJ kg^{-1})	13.56	12.38	25.22	8.11	19.99

[a]Calculated by difference.

Table 5. The C content (%) in Different Phases in the Presence of Different Catalysts

	Catalysts				
Phases	ZrO_2/SO_4^{2-}	HZSM-5	Blank	MgO/MCM-41	KtB
Bio-oil phase	47.19	51.60	63.97	60.50	79.21
Gas phase	3.27	3.39	2.90	2.87	2.38
Aqueous phase[a]	31.12	29.87	13.47	26.28	11.14
Solid phase	18.42	15.14	19.66	10.35	7.27

[a]Calculated by difference.

should be noted that the present percent area values in this study only illustrate the relative concentration of each compound in the fraction of the bio-oil that can be vaporized and passed through the GC column.

As can be seen from Table 6, The main compounds of the bio-oil included hexadecanamide, 3-isopropylidene-5-methyl-hex-4-en-2-one, (2E)-3,7,11,15-tetramethyl-2-hexadecene, 2(4H)-benzofuranone-5,6,7α-tetrahydro-4,4,7α-trimethyl, 2-methyl-2-cyclopentenone, and so forth, for catalytic as well as noncatalytic processes. Chen et al.[17] and Zou et al.[16] used GC-MS to investigate the composition of liquid products from noncatalytic HTL of microalgae. They found that carboxylic acids, acidamides, and ester derivatives were the main components of the obtained bio-oils. Comparison of the main components of the five bio-oils obtain from catalytic HTL over various catalysts revealed that the structure and the acid/base properties play a crucial role in the product composition. When HZSM-5 and MgO/MCM-41 were used as the catalyst, there are a large number of ketone (e.g., 2-methyl-2-cyclopentenone, 2-cyclohexen-1-one-5-methyl-2-(1-methylethyl) and 3-isopropylidene-5-methyl- hex-4-en-2-one), alkene (e.g., 1-pentadecene, naphthalene-1,2,3,4-tetrahydro-1,1,6-trimethyl and (2E)-3,7,11,15- tetramethyl-2-hexadecene) and alkane (e.g. 3,7,11,15-4-methyl-5-olefinic-cetane) while very little carboxylic acids. Therefore, the stability and combustibility of bio-oil will be improved greatly. Especially for HZSM-5 catalyst facilitates cracking reactions to convert the heavier components of the bio-oil into smaller fuel-range molecules. These results may be related to the structure of the HZSM-5 and MgO/MCM-41, the further influence mechanism is beyond the scope of this article, but it could be accomplished in the future work.

Compared with HZSM-5 and MgO/MCM-41, the content of carboxylic acid is very significant when ZrO_2/SO_4^{2-} and KtB were used as the catalyst as well as the blank value. Table 7 lists the relative percentages of major compound classes identified by GC-MS in the bio-oil obtain from catalytic HTL. The content of carboxylic acid is up to 64.90 wt % when KtB was used as the catalyst. This may be related to the destruction of microalgae cell wall structure and the hydrolysis of lipid under the strong base conditions.[25] Meanwhile, the compound category in the bio-oil are significantly reduced when KtB as the catalyst because of the condensation reaction.

Interesting, previous studies also found that acid-catalyzed is advantageous to the extraction of lipids in the microalgae,[38] and the extracted lipid can be converted into carboxylic acid via acid hydrolysis. Therefore, a significant content of carboxylic acid can be observed in the bio-oil when ZrO_2/SO_4^{2-} was used as the catalyst. In addition, ZrO_2/SO_4^{2-} as the catalyst resulted in a lower bio-oil yield and much smaller molecule compounds in the bio-oil due to the acid cracking reaction.[38]

Catalytic HTL mechanism

Since the main components of *D. tertiolecta* are made up of proteins, carbohydrates, and lipids, the mechanisms in the HTL process are very complex in both the catalytic and noncatalytic process, and numerous of organic compounds can be indentified in the bio-oil.[9,13,17,25] Therefore, it is very

Table 6. GC-MS Results of the Bio-Oil Obtained from Catalytic HTL over Various Catalysts

			Relative peak area/%				
No.	Retention time/min	Organic compounds	ZrO_2/SO_4^{2-}	HZSM-5	Blank	MgO/MCM-41	KtB
1	4.17	2-Methyl-2-cyclopentenone	2.07	8.38	2.59	7.66	1.83
2	9.42	3-Isopropylidene-5-methyl-hex-4-en-2-one	1.13	7.11	2.66	10.40	1.67
3	9.82	2-Methyl-5-hydroxypyridine	[a]	[a]	3.59	[a]	[a]
4	11.88	2-cyclohexen-1-one-5-methyl-2-(1-methylethyl)	[a]	4.59	2.16	6.53	1.53
5	14.62	1,1,6-Trimethyl-1,2-dihydronaphthalene	[a]	1.85	[a]	[a]	[a]
6	14.71	Naphthalene-1,2,3,4-tetrahydro-1,1,6-trimethyl	[a]	3.65	1.66	4.65	[a]
7	17.94	1-Pentadecene	[a]	8.43	3.69	8.72	3.03
8	18.14	Pentadecane	12.71	[a]	[a]	[a]	4.25
9	19.07	2(4H)-Benzofuranone-5,6,7α-tetrahydro-4,4,7α-trimethyl	5.87	16.73	9.60	20.90	6.29
10	23.84	DL-Alanyl-l-leucine	7.44	[a]	5.75	[a]	[a]
11	25.58	3,7,11,15-4-Methyl-5-olefinic-cetane	[a]	12.63	[a]	14.00	[a]
12	25.85	(2E)−3,7,11,15-Tetramethyl-2-hexadecene	2.80	13.53	3.12	10.55	4.11
13	25.96	4- Methyl-1-tridecene	6.70	[a]	6.11	[a]	[a]
14	28.06	7-Methyl-9-olefinic undecanoate	11.05	[a]	8.73	[a]	[a]
15	28.49	n-Hexadecanoic acid	33.74	[a]	41.34	12.51	49.95
16	27.55	8-Twelve alcohol	[a]	5.41	[a]	[a]	[a]
17	27.64	7-Methyl-9-olefinic-ondecanol	[a]	4.16	[a]	[a]	[a]
18	27.84	4-Methyl-1,9-diene-tridecane	[a]	9.36	[a]	[a]	[a]
19	31.59	Oleic Acid	[a]	[a]	[a]	[a]	14.94
20	31.72	9,12-Octadecadienoic acid (zz)	6.27	[a]	[a]	[a]	[a]
21	31.94	3-Benzyl-6-methyl-2,5-piperazinedione	2.27	[a]	[a]	[a]	[a]
22	32.32	Hexadecanamide	1.00	4.16	3.47	4.88	2.23
23	33.50	3-Benzyl-6-isopropyl-2,5-piperazinedione	4.19	[a]	[a]	[a]	[a]
24	37.64	3-Hydroxybutyl-quinoline	[a]	[a]	2.16	[a]	2.94
25	46.58	Vitamin E	2.04	[a]	3.37	[a]	[a]

[a]No major compounds (>1% of TIC) were identified

Table 7. Relative Percentage of Major Compound Classes Identified by GC-MS in the Bio-Oil Obtained from Catalytic HTL

Catalyst types	Acids (wt %)	Ketones (wt %)	Alkanes (wt %)	Alkenes (wt %)	Alcohols (wt %)	Esters (wt %)	Aromatics (wt %)	Others (wt %)
ZrO_2/SO_4^{2-}	47.45	9.07	13.71	9.50	11.05	1.13	6.46	2.04
HZSM-5	–	32.22	34.58	18.12	9.57	3.65	1.85	–
Blank	47.09	14.85	7.16	11.39	–	1.66	5.75	3.37
MgO/MCM-41	12.51	38.16	27.61	17.08	–	4.66	–	–
KtB	64.90	10.42	16.10	5.64	–	–	2.95	–

– No organic compounds (>1 % of TIC) were identified

difficult to determine the details of the liquefaction mechanism clearly.[39] So far, although many efforts have been paid to investigating the liquefaction pathway,[14,17,39,40] the real liquefaction mechanism still remains uncertain yet. The water in the present of HTL serves as both solvent and reactant to hydrolytically decompose the proteins, lipids, and carbohydrates.[13] The introduction of catalyst may influence the liquefaction process to some extent.

The obtained gas products were direct determined via GC-MS analysis. According to the analysis results, CO_2 and CO, which are derived from decarboxylation and decarbonylation, and so forth, are the main gas product of HTL, the rest of the gas fraction constituted of low amounts of CH_4, N_2, H_2, C_2H_4, C_2H_6, and so forth. Our experimental results showed that the amount of the gaseous product is less than 5% when the volumetric loading ratio of algal feedstock and water in reactor is 80% during HTL microalgae. Therefore, the output of gas products, which is limited in the evaluation of HTL reaction mechanism, can be negligible.

The aqueous phase was pretreated by SPME and DAE, and the obtained organic products can be determined directly via GC-MS analysis. The analysis results indicated that main products in the aqueous phase include ketones, acids, pyridine, alcohol, phenol, indole, and so forth. These products are derived from the compounds in bio-oil phase via many further reactions include hydrolysis, depolymerization, repolymerization/self-condensation, rearrangement, dehydration, and so forth. Compared with composition of bio-oil phase, the composition of aqueous phase is very limited for the contribution of HTL reaction mechanism. Therefore, in this article, our main work is mainly focus on the composition of bio-oil phase, and we would like deduce a plausible reaction mechanism of *D. tertiolecta* on the basic of the obtained experimental results from bio-oil fraction and the references.[14,17,39,40]

Some typical conversion processes is described in Figure 7. Carbohydrates, which are an important component in the algal cell, can be converted into glucose via hydrolysis. Then, glucose can be further transformed via some reaction including dehydration, decomposition, Maillard reaction, condensation, rearrangement, and so forth.[39] For example, 2-methyl-2-cyclopentenone, which is an important product in the bio-oil obtained from both catalytic and noncatalytic HTL in our study, may be obtained from glucose via a series of chemical conversions, as represented in Figure 7A. The whole process can be described as several sequential procedure as follows: (1) glucose underwent dehydration to form unsaturated conjugate aldehyde under the condition of H^+; (2) and then intramolecular rearrangement reaction occurred, result in the formation of enol form, which can be converted into ketone via another rearrangement reaction; (3) the intramolecular aldol condensation reaction took place, and final product 2-methyl-2-cyclopentenone was obtained.

The conversion of the other two main components, lipids and proteins, may include the routes revealed in Figure 7B. Similar to carbohydrates, both lipids and proteins are activated by hydrolysis reaction.[13,17] For protein, two molecule amino acids derived from the hydrolysis of proteins underwent cyclodimerization to generate cyclodipeptide which only exists in the bio-oil with ZrO_2/SO_4^{2-} as the catalyst. Amino acids molecule also underwent deamination to generate small molecular carboxylic acids and ammonia, and the ammonia can react with fatty acids derived from the hydrolysis of lipids to form acidamides.[17] Meanwhile, fatty acids

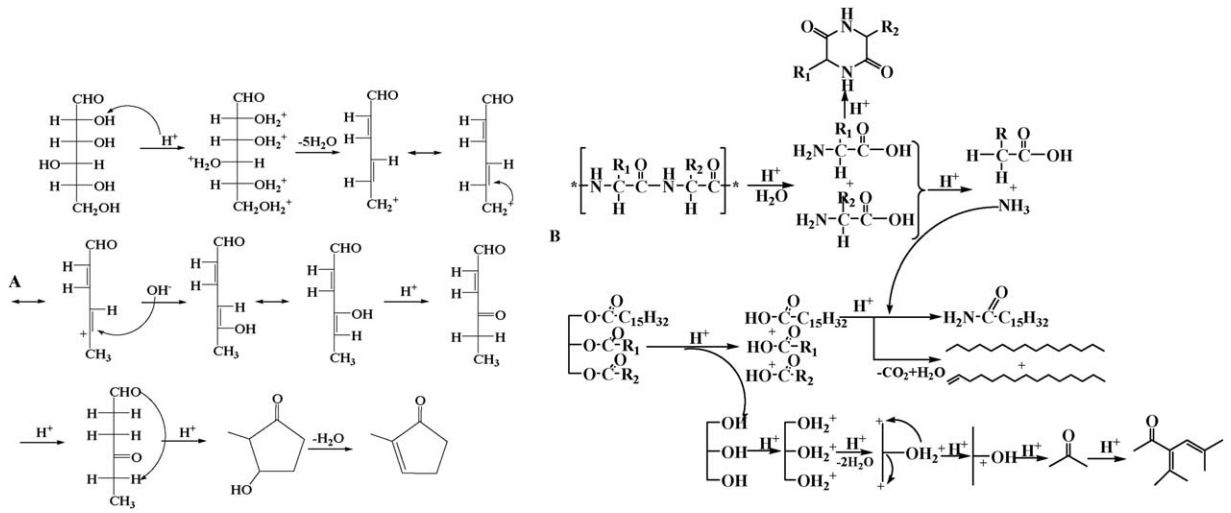

Figure 7. Probable catalytic HTL mechanisms of typical compounds in the microalgae (A): Carbohydrates; (B): Proteins and lipids.

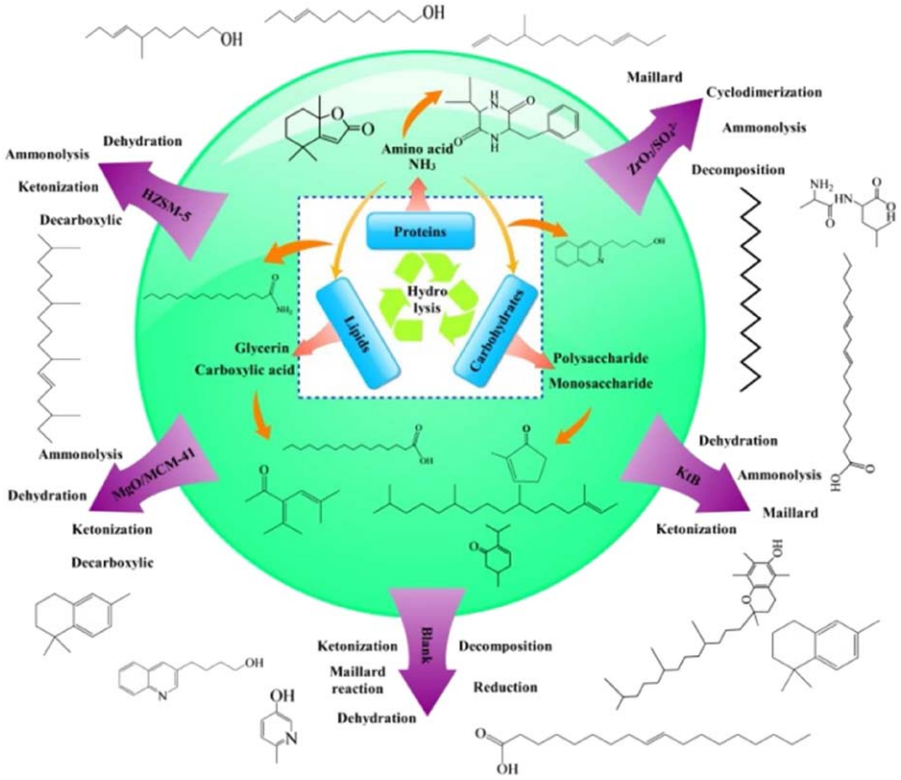

Figure 8. Catalytic HTL microalgae model over various catalysts.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

can be converted into alkanes and/or alkenes via decarboxylic and dehydration.[17] In addition, the other product glycerol can undergo dehydration, ketonization and condensation to form corresponding ketones compounds under the condition of acid. Others reactions, such as amination, reduction, decomposition, and substitution, may also take place in the both catalytic and noncatalytic HTL.

According to the mechanism mentioned before, the probable catalytic HTL microalgae model over various catalysts can describe the catalytic HTL reaction simply (Figure 8). As can be seen from Figure 8, the main components in the microalgal cell start from hydrolysis reaction to form numerous intermediate products, proteins to amino acids, ammonia, peptides, and so forth; carbohydrates to monosaccharides and oligosaccharides; lipids to glycerol and carboxylic acids. Then, these intermediate products may react with each others in the absence/presence of catalyst. In general, when HZSM-5 and MgO/MCM-41 were used as the catalyst, the main chemical reactions include ketonization, decarboxylic, dehydration, ammonolysis, and so forth. The cyclodimerization, decomposition, Maillard reaction and ketonization are the main reactions with ZrO_2/SO_4^{2-} as the catalyst. The dehydration, ammonolysis, Maillard reaction, and ketonization can occur with KtB as the catalyst. In the absence of catalyst, the reactions had no obvious regularity. Most of reactions can be found in the blank.

Unfortunately, the mechanism and model mentioned above all only based on the GC-MS results of bio-oil and only some of reaction routes can be deduced while a large number of actual conversion mechanisms are still ambiguous due to the lack of the intermediate information. ^{13}C isotopic tracer method and other *in situ* technologies may provide detailed information for illuminating the HTL mechanism, systematic study may be carried out in further work.

Conclusions

Based on the results presented above, some conclusions can be drawn as follows:

1. According to the conversion and bio-oil yield, the catalytic performance of the used catalysts follows the trend of KtB > MgO/MCM-41 > Blank > ZrO_2/SO_4^{2-} > HZSM-5. When KtB was used as the catalyst, both the conversion and bio-oil yield are up to the optimal values, 94.84 and 49.09 wt %, respectively.

2. The analysis results affirmed that the addition of catalyst is beneficial to reduce the fixed carbon content in the bio-oil, and catalyst structure has a certain influence on the composition and boiling point distribution of bio-oil. Although the type of composition in the bio-oil obtained from HTL over four kinds of catalyst is very similar, the dominating content of composition is remarkably different.

3. The obtained bio-oil represented low acid content and high hydrocarbon content using HZSM-5 and MgO/MCM-41 as the catalyst, therefore, the quality of bio-oil is relatively better than others.

4. Based on our results and previous studies, the probable catalytic HTL microalgae model over various catalysts can be described that the main chemical reactions include ketonization, decarboxylic, dehydration, ammonolysis, and so forth. with HZSM-5 and MgO/MCM-41 as the catalyst; the cyclodimerization, decomposition, Maillard reaction, and ketonization are the main reactions with ZrO_2/SO_4^{2-} as the catalyst; the dehydration, ammonolysis, Maillard reaction, and ketonization can occur with KtB as the catalyst.

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