Petroleum and Coal

Article **Open Access**

ACID IONIC LIQUID [HMIM]HSO_4 AS CATALYST FOR THE PRODUCTION OF BIODIESEL FROM MICROALGAE

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Received September 18, 2017; Accepted November 16, 2017

Abstract

The reaction conditions of transesterification during *Chlorella protothecoides* biodiesel preparation using an acid ionic liquid [Hmim]HSO_4 as catalyst was studied. The product was analyzed by gas chromatography and the productivity was calculated. The results showed that under ultrasonic condition, ethyl ether had the highest extraction rate (9.97% at 25min). The best conditions of transesterification were as follows: the molar ratio of methanol to the microalgae oil was 9:1, the reaction time was 7h, the reaction temperature was 70 ºC and the dosage of the catalyst accounted for 7% of the mass of the microalgae oil. Under this condition, the yield of biodiesel could reach 64%~65%. As a result, the production had already reached the diesel standard of China from the perspective of physical and chemical properties.

Keywords: Ionic liquid; biodiesel; microalgae; transesterification; *Chlorella protothecoides*.

1. Introduction

With the increasingly depletion of oil deposit worldwide and the attention paid to public environment pollution, global countries endeavor to find useful and renewable alternative fuel. In recent years, biodiesel has drawn public attention due to its advantages such as renewability, comparable combustibility to traditional petroleum and low pollution of combustion tail gas. Compared to fossil fuel, the major component of biodiesel is fatty acid methyl ester, including hydrogen, oxygen and carbon elements, it generates much lower toxic air emissions when it burns. Virtually, biodiesel does not contain sulfur element and nitrogen element, leading to zero nitric oxide and sulfur dioxide emissions; and biodiesel is an oxygenated fuel, which could increase the rate of fuel combustion and reduce the production of carbon monoxide and soot. In addition, biodiesel has more advantages such as wide sources of the raw materials, low processing cost, high calorific value, and stable performance. Based on the above characteristics, biodiesel is recognized to be an ideal substitute for petroleum diesel and has extensive application prospects. Traditionally, it could be prepared from vegetable oils, animal fats and waste cooking oil with short chain alcohol through the transesterification of triglycerides in the presence of a catalyst. Since 1988, many European countries has started to use biodiesel as a substitution of traditional diesel [1]. As the main factors restricting large-scale production of biodiesel, the question of feed stock cost [2] and conversion rate has always been research emphasis in this area.

Algae has the characteristics of high efficient of photosynthesis, high environment adaptability, high production and short life cycle [3-4]. Thus, algae is an ideal material of biodiesel. *Chlorella protothecoides.* is one strain of green algae that can grow autotrophically or heterotrophically under different nutrition conditions in culture medium with various chemical content. Hetero-trophic growth can accumulate high content of lipid within cell. In addition to improving growth efficiency, heterotrophic growth can also accumulate other metabolites such as protein that help to lower the cost of biomass and lipid production [5].

Biodiesel is normally prepared by transesterification reaction. At present, the transesterification methods include homogeneous acid and base catalytic method, heterogeneous catalytic method, enzyme catalytic method and supercritical method [6-7]. Among them, homogeneous acid and base catalytic method is more mature than the other methods with its' high reaction efficiency, high yield and industrial application. However, strong acid and base catalysts have great corrosivity on equipment. Additionally, the waste produced during reaction is not only harmful to environment but also is hard to be separated and recovered. Heterogeneous catalytic method is very potential and promising in the future industrial application due to many advantages compared to homogeneous catalytic method such as easy to be recovered, reusable and mild reaction condition [8]. Ionic liquid is a series of new solvent and catalyst. In recent years, as environmental friendly catalyst, ionic liquid is gradually used in the research of biodiesel production. But there are still many restrictions for mass production like high cost of catalyst, inactivation and so on. To find a catalyst with high efficiency and low cost is always the emphasis in this biodiesel production field.

2. Materials and methods

2.1. Materials

Chlorella protothecoides, powder with about 13% of lipid bought from Shandong Wuli Lvqi Biological Engineering Company; N-methyl imidazole (purity ≥98%) and concentrated H_2SO_4 (AR) from Beijing Chemical Plant; Disodium phosphate, sodium methoxide, vanadium pentoxide, etroleum ether (AR, boiling range: 60-90 °C), methyl ether (AR) and n-hexane (AR) from Tianjin Fuyu Chemical co., Ltd; Absolute methanol bought from Tianjin Dengke Chemical Reagent Co., Ltd; Methyl heptadecanoate internal standard and fatty acid methyl ester profiling mix bought from J&K Chemical.

2.2. Methods

2.2.1 Preparation of ionic liquid

Added 8.2g N-methyl imidazole (719 mL, 011 mmol) into a 250mL three-necked bottle with mechanical stirring. And then put this three-necked bottle into cold water to cool to 0~5°C. Added mixture of 10.2g 98% concentrated sulfuric acid and 10mL water within 30 minutes and kept stirring 2 hours under room temperature. Removed water by reduce pressure distillation under 75 °C and got colorless and transparent ionic liquid $[Hmim]HSO_4$ 17.5g. The yield was 98.2% [9].

2.2.2. Extraction of Chlorella protothecoides lipid

Put 5g dry *Chlorella protothecoides* powder into a conical flask containing a certain extraction agent and extracted lipid under ultrasonic condition. After extraction, centrifugalized for 10 minutes under condition of 3500r/min. Collected supernatant and separated by reduced pressure distillation. Dried to constant weight under 50°C and calculated extraction rate as the following formula.

$$\text{Extraction rate} = \frac{Mass\ of\ lipid}{Mass\ of\ \ dry\ Chlorella\ \text{powder}} \times 100\%$$

2.2.3. Preparation of biodiesel

Took 10g of *Chlorella protothecoides* lipid into a 250mL round-bottom flask, added a certain amount of methanol and catalyst and reflux reacted under hydrothermal condition. Stopped heating and stirring after reaction several hours. Put mixture into a separating funnel and stratified statically. The bottom layer was ionic liquid layer and ionic liquid could be reused after separation; the upper layer was product layer and biodiesel could be separated after removing methanol, water and glycerin by reduced pressure distillation. Biodiesel product was analyzed chromatographically then.

2.3. Analysis of product

Analyzed products using SP6800A gas chromatography by internal standard method [10]. Injected 1μL of biodiesel product into a 1ml volumetric flask, weighed the mass of the injected product and recorded it. Then diluted with n-hexane after adding 1mg internal standard. Sampled this solution 1μL with micro-syringe and analyzed with chromatography. Calculated conversion rate using the following formula:

$$\text{Yield} = \frac{\textit{Total peak area of FAME}}{\textit{Peak area of internal standard}} \times \frac{\textit{Mass of internal standard}}{\textit{Mass of biodiesel product sample}}$$

2.3.1. Determination of biodiesel's properties

Tested the density, viscosity, moisture content, sulfated ash content and copper corrosion degree of *Chlorella protothecoides* biodiesel prepared in this research and compared them to Chinese standard to make sure whether it meets the basic requirements of petroleum products in China.

3. Results and discussion

3.1. Extraction efficiency effect of different extraction agents and extraction time under ultrasonic condition

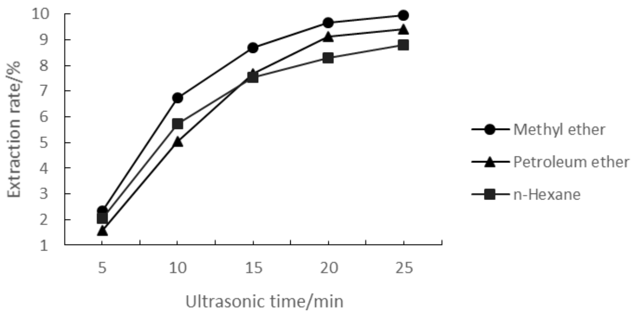

Figure 1. Extraction efficiency effect of different extraction agents and extraction time under ultrasonic condition

Under ultrasonic condition, the extraction efficiency of *Chlorella protothecoides* lipid rises with the increasing of extraction time at first. After 20 minutes, rising rate of extraction efficiency gradually declines and finally it is stable at 9%. No obvious differences are found of three different extraction agents on extraction efficiency. Among these three agents, extraction rate of ethyl ether reaches the highest 9.97% at 25 minute, followed by petroleum ether(9.43%) and n-hexane (8.79%) (Fig.1). Ethyl ether has a strong capacity of lipid dissolution rate. However, its' low boiling point, feature of inflammable, containing 2% of water in saturated solution, as well as easy to extract ingredients that is not lipid such as carbohydrate make it no best choice in the research.Comparably, petroleum ether is not inflammable and it allows trace amount of water when utilizing [11]. Thus, considering various factors, in this experiment, all lipid was extracted by petroleum ether under ultrasonic condition at the length of 25 minutes.

3.2. Effect of the molar ratio of methanol to oil on biodiesel yield

This experiment studied the effect of the molar ratio of methanol to oil on biodiesel yield under the condition of reaction time 7h, reaction temperature 70°C and catalyst dosage 7%. The yield of biodiesel rises as the molar ratio of methanol to oil increasing. When this ratio comes to 9:1, the conversion rate reaches the highest 65.4%. But conversion rate drops when more methanol is added into reaction system.

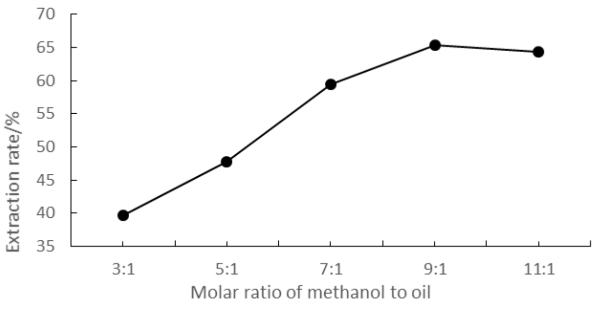

Figure 2. Effect of the molar ratio of methanol to oil on biodiesel yield

When molar ratio is 3:1, reaction is not complete and the reaction equilibrium coefficient inclines left. Therefore, little methyl ester is found in the produced biodiesel. Excessive reactant in a certain reaction can help the equilibrium coefficient shift right. Thus, the higher molar ratio of methanol to oil is, the higher the reaction conversion rate is, and also the shorter the time that reaction will reach equilibrium. While the content of methanol is low, adding of methanol into reaction system can accelerate positive reaction which increases final conversion rate.However, continuing increasing of molar ratio will lead that the content of methanol in reaction system makes little contribution to positive reaction. Consequently, no obvious change of reaction rate is found. Additionally, more reactant will impose burden on production separation and recovery step. One other explanation is that, because of the good solubility of methanol on ionic liquid, more methanol is beneficial to the dispersion of ionic liquid in reaction system that will produce more contact between ionic liquid and lipid. In this respect, the adding of excessive methanol will improve the final conversion rate of biodiesel. But continuous increase of methanol's dosage will dilute the concentration of catalyst which lead a decrease of biodiesel yield instead [12].

3.3. Effect of reaction time on biodiesel yield

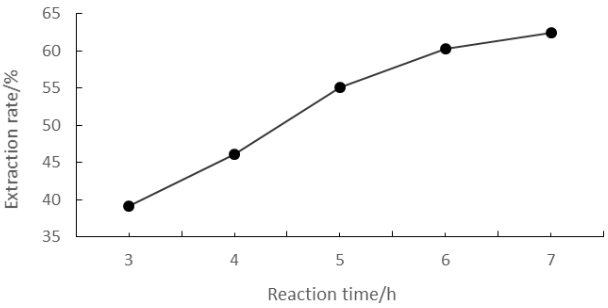

Figure 3. Effect of reaction time on biodiesel yield

This experiment studied the effect of reaction time on biodiesel yield under the condition of reaction temperature 70°C, catalyst dosage 7% and molar ratio of methanol to oil 9:1. Fig. 3 shows that biodiesel yield rises with the increase of reaction time and it gradually goes down after reacting 6 hours. At the beginning of reaction, low contact between methanol and oil lead a low reaction rate. This reaction rate keeps increasing till reaction equilibrium. From then on, more reaction time will not only have no benefit on the right shift of reaction,but also increase many side reactions such as decomposition and polymerization that may affect oil's quality. Therefore, by comprehensive consideration of factors like efficiency, cost and quality of product, this experiment takes 7 hours as reaction time. At this point, the biodiesel conversion rate is 62.4%.

3.4. Effect of the reaction temperature on biodiesel yield

This experiment studied the effect of reaction temperature on biodiesel yield under the condition of reaction time 7h, catalyst dosage 7% and molar ratio of methanol to oil 9:1. Result reveals that under low temperature (50°C), the conversion rate is very low (about 41.4%). And with the rising of reaction temperature, biodiesel yield reachs the highest 65.4% at 70°C.

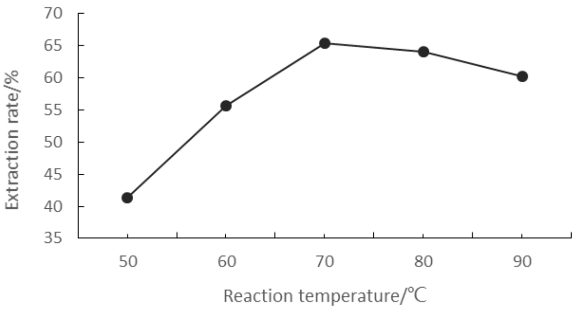

Figure 4. Effect of the reaction temperature on biodiesel yield

Reaction time is a key influence factor of transesterification during biodiesel production. Too low or too high of reaction temperature is bad for the conversion of oil. This is because that increase of reaction temperature will decline the separation of differrent phases that improves the mixture of materials. However, too high temperature will decrease methanol's concentration in reaction system due to methanol's vaporization. Then the contact between lipid and catalysts is lowered, and so as the biodiesel conversion rate. Fig.4 shows that conversion rate is only 60.2% when reaction temperature is 90°C. Additionally, high reaction time may produce many side products such as dimethyl ether and glyceryl ether. This effect will interference the next processing of product.

3.5. Effect of catalyst dosage on biodiesel yield

This experiment studied the effect of catalyst dosage on biodiesel yield under the condition of reaction time 7h, reaction temperature 70°C and molar ratio of methanol to oil 9:1.

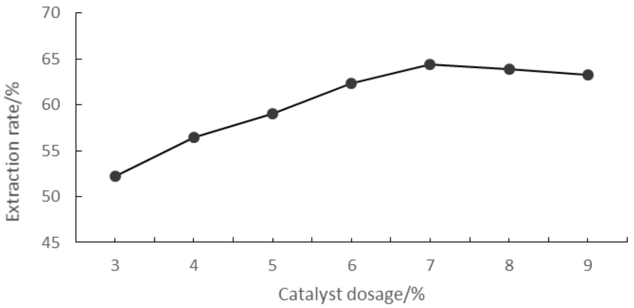

Figure 5. Effect of catalyst dosage on biodiesel yield

Fig.5 demonstrates that biodiesel yield rises as catalyst dosage increasing. When catalyst dosage is 3%, conversion rate is only 52%. And it reaches 64.4% when the amount of catalyst accounts for 7% of the oil. But keeping adding of catalyst does no positive contribution to biodiesel yield (63% when catalyst dosage is 9%). This may because that excessive catalyst will influence its' scatter condition in methanol-oil system.

Therefore conversion rate declines due to decrease of contact area between catalyst and oil. On the other hand, because of increase of catalyst, at late stage of reaction, content of *Chlorella*'s *protothecoides* lipid will relatively drop which is bad for transesterification reaction. Besides, during experiment, with the increase of catalyst dosage, product's color gets darker. This may because some ions go into product system that imposes more burden on separation and purification of product. Hence 7% catalyst dosage is the best choice during transesterification reaction in this experiment.

3.6. Effects of heteropoly ionic liquids

Based on the results of the former experiments, the suitable reaction condition by using acid ionic liquid [Hmim]HSO$_4$ as catalyst were as follows: molar ratio of methanol to oil 9:1; reaction time 7h; reaction temperature 70°C; catalyst dosage 7%. Under these conditions, the catalytic effects of heteropoly ionic liquids were tested. Table. 1 showed that the yields of biodiesel when using three types of heteropoly ionic liquid are 69.4%, 72.2% and 75.0%, respectively, which are all higher than the catalyst [Hmim]HSO$_4$.

Heteropoly anion can form a cage-like tertiary structure similar with zeolite with hydrated proton. Nonpolar molecules can react at the solid surface of heteropoly anion. Polar molecules not only can react at the surface, but also can goes into the inner structure of heteropoly molecule and react. At this time, heteropoly chemicals are something like solution that are in favor of the transfer of react molecules. Besides, the acidity of heteropoly chemicals is higher the traditional mineral acid such as H_2SO_4, HCl and HNO_3. This is also a reason why the catalytic effect of heteropoly ionic liquid is higher than [Hmim]HSO$_4$. Among these three heteropoly ionic liquids, [Hmim]$_6$PMo$_9$V$_3$O$_{40}$ has the highest conversion rate of biodiesel [13]. This is because the acidity of these three heteropoly anions are increasing as the molar number of vanadium increases. Therefore the catalytic efficiency of these three heteropoly ionic liquids increases as the molar number of vanadium increases.

Table 1. Yields of biodiesel by different catalysts

Catalyst	Yield
[Hmim]HSO4	64.4%
[Hmim]4PMo11VO40	69.4%
[Hmim]5PMo10V2O40	72.2%
[Hmim]6PMo9V3O40	75.0%

3.7. Properties evaluation of biodiesel production

The biodiesel production was made under the condition of reaction time 7h, reaction temperature 70°C, molar ratio of methanol to oil and molar ratio of methanol to oil 9:1. Fatty acid methyl esters in the biodiesel prepared by *Chlorella protothecoides* are shown in Table 2. Its' physical and chemical properties are illustrated in Table 3.

Table 2 Fatty acid methyl esters in the biodiesel prepared by Chlorella protothecoides

No	Molecular formula	Relative molecular mass	Fatty acid methyl ester	Relative content (wt%)
1	$C_{15}H_{30}O_2$	242	Methyl tetradecanoate	5.21
2	$C_{17}H_{34}O_2$	270	Hexadecanoic acid methyl ester	17.74
3	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic acid methyl ester	21.22
4	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid methyl ester	45.67
5	$C_{19}H_{38}O_2$	298	Octadecanoic acid methyl ester	6.23
6	$C_{20}H_{38}O_2$	310	10-Nonadecenoic acid methyl ester	3.93

Table 3. Physical and chemical properties of biodiesel production

Properties	Biodiesel product	Chinese requirement
Density/g·cm-3	0.860	0.820~0.900
Viscosity/$mm^2 \cdot s^{-1}$	5.3	1.9~6.0
Moisture content/%	0.001	≤0.05
Sulfated ash content /%	0.013	≤0.020
Copper corrosion degree	1	≤1
Flash point (°C)	107	75
Acid value (mg KOH/g)	0.349	Max 0.5
Heating value (MJ/kg)	40	40–45
H/C ratio	1.81	1.81

The information of Table.3 shows that the density, viscosity, moisture content, sulfated ash content and copper corrosion degree of *Chlorella protothecoides* biodiesel prepared in this research are not only in line with Chinese requirements about petroleum product, but also are equal to traditional petroleum [14-15]. It can be used in engine as substitute of traditional petroleum and also can be used mixed with traditional petroleum to lower the cost.

4. Conclusions

Compared to n-hexane and ethyl ether, petroleum ether was the best choice of extraction agent. In this research, the highest extraction rate of *Chlorella protothecoides* lipid was 9.97%. The suitable reaction conditions found to produce *Chlorella protothecoides* biodiesel by transesterification reaction using acid ionic liquid [Hmim]HSO_4 as catalyst were as follows: molar ratio of methanol to oil 9:1; reaction time 7h; reaction temperature 70 °C; catalyst dosage 7%. Under these condi-tions, the biodiesel conversion rate was about 64-65%. The product met China's require-ments of petroleum products on physical and chemical qualities and it could be applied practi-cally. Due to low content of lipid in the chosen *Chlorella protothecoides* in this research, in the future, more research about improving lipid content of *Chlorella protothecoides* should be done like transgenic technology or cultural condition optimization to increase conversion rate and lower cost.

Acknowledgement

Financial support from the Scientific Innovation Program of Shenzhen City, China, under basic research program (JCYJ20150626095215791), is gratefully acknowledged.

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