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Liquefaction and characterization of residue of oleaginous yeast in polyhydric alcohols

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Abstract−The residue of oleaginous yeast (ROY) was liquefied in polyhydric alcohols using sulfuric acid as catalyst. The effects of some liquefaction conditions on the liquefied residue rate, such as liquefaction temperature, catalyst loading, reaction time, glycerol concentration and solvent/ROY ratio, were discussed. The liquefied residue rate decreased as the reaction time, liquefaction temperature, catalyst loading, solvent/ROY ratio increased. The re-polymerization of liquefied products was favored in later stage reaction. Higher catalyst loading and lower solvent/ROY ratio could accelerate the re-polymerization of liquefied products; thus the liquefied residue increased. Fourier transform infrared (FT-IR) analyses showed that the main component of ROY is polysaccharide. The gas chromatography and mass spectrometry (GC-MS) analysis showed that liquefied products of ROY included alcohols, acids, ketones, aldehydes, amide, ester and their derivatives.

Keywords: Liquefaction, Oleaginous Yeast, Polyhydric Alcohol, Polysaccharide

INTRODUCTION

Microbial oil, also called as single cell oil, is a triglyceride (lipid) produced from microorganisms, such as yeast or algae, by fermentation. Generally, the oleaginous yeast species may accumulate about 20% lipids by mass fraction of their biomass [1]. Solvents extraction is usually used for lipid separation from microorganism and chloroform is identified as the most commonly used solvent [2]. However, about 50% residue by mass fraction of yeast remains after lipid extraction, and these residues have not been utilized effectively. Meanwhile, the production cost of microbial oil from the lignocellulose is relatively high compared to petrochemical fuel. The effective utilization of the residue of oleaginous yeast (ROY) is very important, as it not only reduces the production costs but also solves their disposal problems.

ROY is mainly composed of polysaccharide, and polysaccharide makes up more than 70% of the dry ROY weight [3]. Generally, the methods for lignocellulose conversion to high-added value products include gasification, pyrolysis, and liquefaction. Among these, liquefaction is the most widely used due to relatively lower process temperature as compared to other thermal conversion processes. Biomass can be converted into liquid chemical products by liquefaction [4]. These liquid chemical products have low molecular weight and high reactivity, which could be used as raw materials of some functional polymers such as polyurethane, resins and adhesives [5]. Several solvents have been carried out previously on liquefaction of biomass [6-8]. Fan et al. [9] and Yip et al. [10] carried out liquefaction of biomass using various solvents such as acetone phenol, ethylene glycol, toluene and ethylene carbonate. Lu et al. [11] investigated the liquefaction of bioethanol fermentation residues of reed and corn-stover using ethanol as solvent. In our previous studies [12], we investigated the liquefaction of acid hydrolysis residue of corncob using polyhydric alcohols in the presence of sulfuric acid. The kinetics of liquefaction of wood and its major cell wall components (cellulose, hemicellulose and lignin) were presented and the kinetic parameters were estimated [13].

In this study, we selected ROY for the liquefaction process. The aim was to develop a highly efficient way of transforming ROY into valuable chemicals and to study the influence of the liquefaction conditions on the liquefaction efficiency. Fourier transform infrared spectrometry (FT-IR) was used to investigate the functional groups of the liquefied products. The compositions of liquefied products from ROY were investigated in detail by GC-MS.

MATERIALS AND METHODS

1. Materials

The residue of oleaginous yeast (ROY) was supplied by ZHONGKE New Energy Co., Ltd. (Yin-kou, China), which was prepared by chloroform extraction of lipid. The elemental composition data of ROY is presented in Table 1. The ROY sample was milled in a knife

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Table 1. Elemental composition of residue of oleaginous yeast

Items	C	H	N	O	S	Ash
wt%	42.85	6.358	2.33	35.737	0.715	12.01

mill to pass through a 165-μm sieve. Polyethylene glycol 400 (average molecular weight, 400, PEG 400), supplied by Dow Chemical, and glycerol from Sinopharm Chemical Reagent Co. were used as liquefaction reagents. Sulfuric acid (98%) was used as the acidic catalyst. All other chemicals were reagent grade and used without further purification.

2. Liquefaction Reaction

The ROY was dried at 105 °C for 24 h before use. The mixture of liquefaction solvents (PEG 400/glycerol=4/1, w/w) and catalyst (sulfuric acid) was placed in a three-neck flask equipped with a mechanical stirrer, a reflux condenser and a thermometer. The concentration of catalyst was calculated as weight content (%) based on the amount of the liquefaction solvent. The flask was immersed in an oil bath and preheated at a certain temperature. When the temperature reached reaction temperature, ROY was charged into the flask. Time zero was considered the time at which the ROY was added to the flask. The liquefaction was conducted under constant stirring and refluxing for a certain time. After the reaction, the liquefaction products were diluted with methanol, and stirred for more than four hours. The liquefied residue (LR) was separated by filtration, and then dried in oven at 105 °C to constant weight (12 h) and weighed for the determination of the content of LR; the percentage of LR was calculated with the following equation:

$$\text{LR}\ (\%) = \frac{W_{LR}}{W_{ROY}} \times 100\% \quad (1)$$

where W_{LR} was weight of LR of ROY (oven-dried), g; W_{ROY} was initial weight of ROY (oven-dried), g.

The content of LR was the average value from two replicate samples, and the run would be repeated if the deviation of the value obtained from the average was higher than 5%.

The viscosity of the liquefaction products at different mass ratio of solvent/ROY was measured with a NDJ-1 rotary viscosity meter (Pushen Instruments, Shanghai, China) at 25 °C.

3. FT-IR Spectroscopy

Infrared spectra of the ROY and LR were characterized by Fourier transform infrared spectrophotometer (BRUKER, TENSOR27, Karlsruhe, Germany), in a 4,000-400 cm^{-1} wave number range. Spectral resolution of the spectrometer was 4 cm^{-1}. All measurements used the KBr disk technique.

4. Gas Chromatographic-mass Spectrometric (GC-MS)

The components in the liquefied products were silylated according to Zhang et al. [7], and then analyzed by GC-MS (Agilent, 7890A-5975C, California, USA) using 30 m×0.25 mm×0.25 μm capillary column (REX-5MS). Helium (99.999%) was used as the carrier gas with a constant flow rate of 1.0 mL/min. The oven was programmed with a 10 °C/min increase to a final temperature of 290 °C and held for 15 min. The injection size was 5 μL. The identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST) 2005 library.

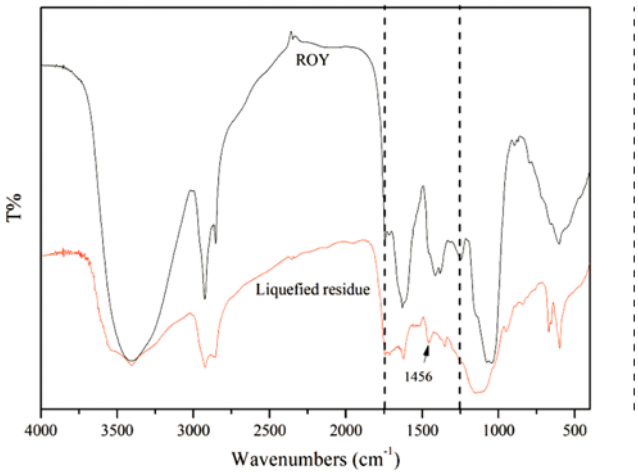

Fig. 1. FT-IR of ROY and its liquefied residue.

RESULTS AND DISCUSSION

Fig. 1 shows the FT-IR spectra of the ROY and its liquefied products. It can be seen that the peaks observed at 3,410 cm^{-1}, 2,925 cm^{-1}, 1,629 cm^{-1}, 1,382 cm^{-1}, 1,074 cm^{-1} and 893 cm^{-1} should be the characteristic absorption bands of polysaccharide [14]. A broadly stretched intense peak at around 3,410 cm^{-1} was the characteristic absorption of hydroxyl group. The strong absorption peak at around 1,741 cm^{-1} and a weak one at near 1,382 cm^{-1} were detected and were indicative of the presence of carboxyl groups. Specifically, the peaks at 1,743 cm^{-1} and 1,247 cm^{-1} confirmed the presence of o-acetyl ester [12]. The IR absorptions at 1,074 cm^{-1} and 1,047 cm^{-1} were also detected and were related to functional groups of pyranglycosides [15]. The absorption occurring at about 893 cm^{-1} shows the β configuration of the glucan linkages [16]. These observations further confirmed that the ROY was composed of polysaccharide.

The FT-IR spectrum of the LR of ROY (Fig. 1) showed that the bands at 1,413 cm^{-1}, 1,382 cm^{-1}, 1,247 cm^{-1}, 1,074 cm^{-1}, 1,047 cm^{-1} and 893 cm^{-1} had disappeared, which indicated that the polysac-

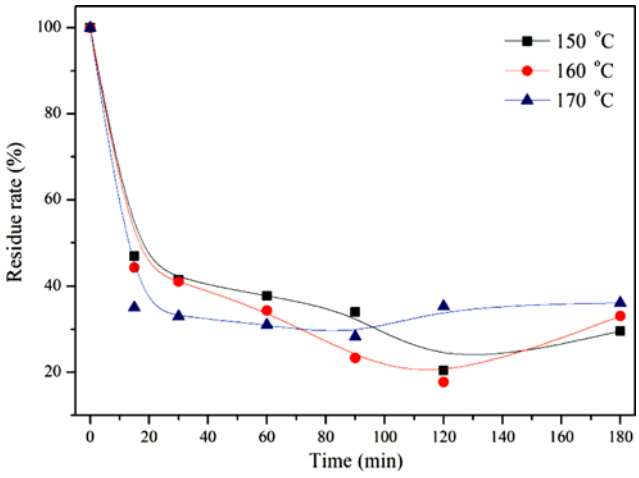

Fig. 2. The effect of reaction time on the liquefied residue rate of ROY (Catalyst concentration, 3%; PEG 400/ROY, 4/1).

charide was liquefied and converted into soluble substance. It was clear that the bands of carbonyl groups at 1,741 cm^{-1} were still found. Furthermore, the bands at 1,456 cm^{-1} and 1,352 cm^{-1} were detected and attributable to carbohydrate, which indicated that the LR would mainly contained some carbohydrate or carbohydrate derivatives.

The time-dependence of ROY liquefaction in polyhydric alcohols at different reaction liquefaction is shown in Fig. 2. ROY was liquefied very rapidly in the first 15 min at all the reaction temperature, and about 50% of ROY was converted into soluble substances at 60 min. After that, ROY was liquefied at a much lower rate, reaching a minimum liquefied residue of 17.71% at 120 min (160 °C). The rapid liquefaction stage observed in the first 30 min of the liquefaction process was largely due to the degradation of polysaccharide, which was different from the liquefaction behavior of the lignocellulose. Lignocellulose biomass was mainly composed of cellulose, hemicellulose, and lignin, and the rapid liquefaction stage of lignocellulose was attributed to the degradation of easily accessible biomass components such as lignin, hemicellulose, and amorphous cellulose [12,17]. Note that the LR content increased in later stage of liquefaction reaction, which could be attributed to the re-polymerization of the decomposed polysaccharide. Similarly, some researchers observed an increase of LR after a certain reaction time and explained this phenomenon as a re-polymerization reaction of the liquefied components [18,19]. They also found that glycerol has a significant effect on retardation of liquefied components, especially decomposed lignin [12,18,20].

On the other hand, the content of LR decreased first and then increased with increase of reaction temperature (Fig. 3). The LR reached 21.85%, 20.41%, 17.71%, 35.34% and 43.29% for 140 °C, 150 °C, 160 °C, 170 °C and 180 °C, respectively. Generally, degradation and re-polymerization coexist in the liquefaction of biomass [12]; degradation produces the liquefied products of biomass and reduces the residue rate. Instead, re-polymerization produces insoluble substance and increases the residue content. In this study, degradation was the predominant phenomenon when the reaction temperature was below 160 °C, but at the higher reaction temperature, the re-polymerization of liquefied products was favored, and thus the LR content was increased.

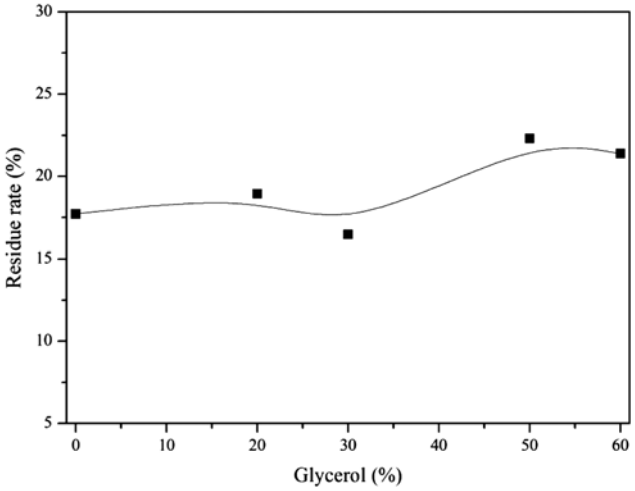

Fig. 4. The effect of glycerol concentration on the liquefied residue rate of ROY (Catalyst concentration, 3%; Polyols/ROY, 4/1; reaction temperature, 160 °C; liquefaction time, 120 min).

To determine whether glycerol can retard the re-polymerization of liquefied products of ROY, we did a series of experiments to evaluate the effect of PEG#400 and glycerol blended on the system; the results are shown in Fig. 4. There was no apparent effect for LR with addition of glycerol to PEG#400 during the liquefaction of ROY, which was contrary to the lignocellulosic biomass liquefaction [19,20].

Liquefaction of ROY with five different mass ratios of solvent/ROY was investigated and the results shown in Fig. 5. It was found that the mass ratio of solvent/ROY apparently affected the liquefaction percentage. The LR content decreased sharply as the mass ratio of solvent/ROY increased from 3 to 4, while the mass ratio of solvent/ROY was more than 4.5; the effect of increasing mass ratio of solvent/ROY became unapparent. The viscosity of liquefaction

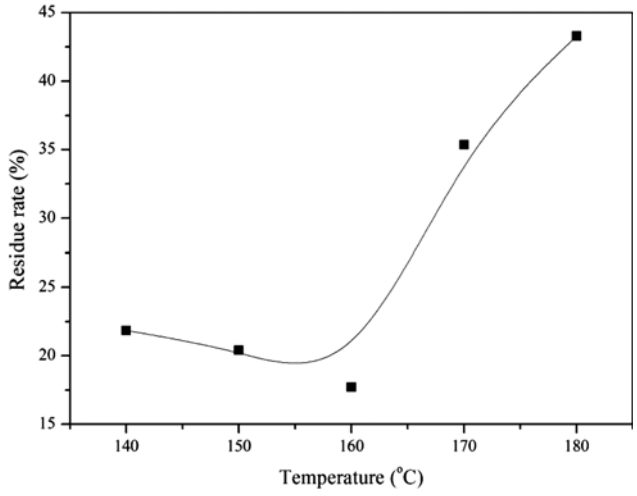

Fig. 3. The effect of reaction temperature on the liquefied residue rate of ROY (Catalyst concentration, 3%; PEG 400/ROY, 4/1; liquefaction time, 120 min).

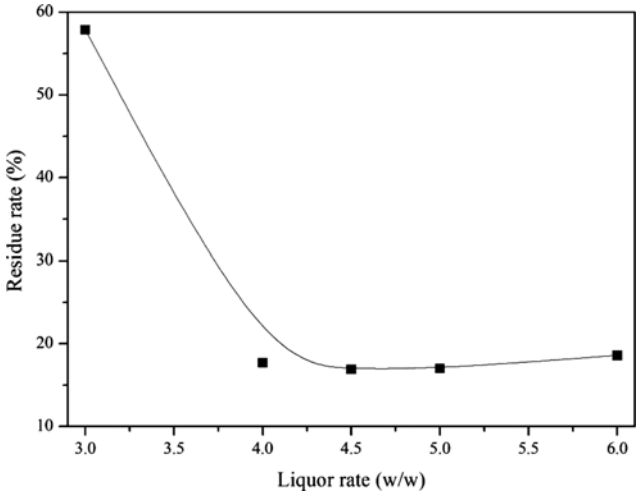

Fig. 5. The effect of mass ratio of solvent/ROY on the liquefied residue rate of ROY (Catalyst concentration, 3%; reaction temperature, 160 °C; liquefaction time, 120 min).

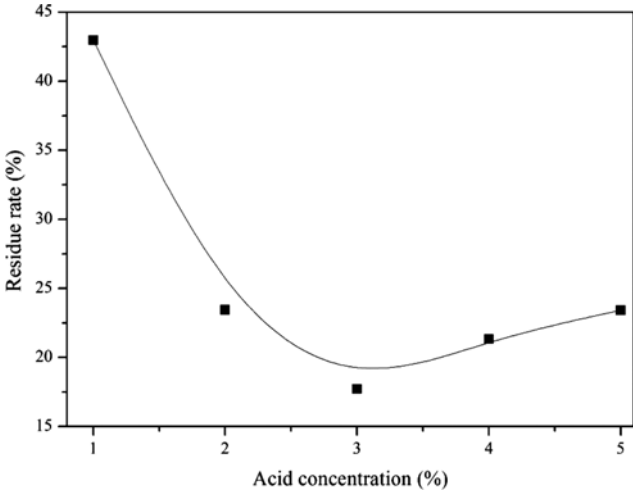

Fig. 6. The effect of acid concentration on the liquefied residue rate of ROY (PEG 400/ROY, 4/1; reaction temperature, 160 °C; liquefaction time, 120 min).

products for the mass ratio of solvent/ROY of 3, 4, 5 and 6 was 1,337 mPa·s, 812.5 mPa·s, 565 mPa·s and 415 mPa·s respectively. Thus, the high LR rate obtained to low mass ratio of solvent/ROY could be attributed to the high viscosity of the liquefaction system, and dissolving capacity of solvent thus decreased when the mass ratio of solvent/ROY decreased. Meanwhile, the re-polymerization reactions of the liquefied components could be accelerated due to the high concentration of liquefied products, and this would break the liquefaction process. On the other hand, less than 20 percent of LR content was obtained with very low quantities of ROY (the mass ratio of solvent/ROY >4) because more liquefaction solvent can dissolve liquefied products and prevent them from re-polymerization at higher liquor ratio. However, high mass ratio of solvent/ROY means that more solvent was used, which could increase the cost of the liquefaction process. Therefore, a mass ratio of solvent/ROY of 4 was enough to get the LR percent as low as 16.89%, which was almost totally liquefied except ash.

Generally, the addition of catalysts significantly accelerated the liquefaction reaction of the biomass. Fig. 6 presents the effect of the acid concentration on the liquefied residue rate of the ROY. The LR content initially decreased as the acid loading from 1 to 3%, and then increased with the further increase in acid concentration, which may be because the acid not only can accelerate the liquefaction reaction, but also enhance the re-polymerization of the liquefied components, with the consequent formation of the liquefied residue. As mentioned, there was a competition between re-polymerization and degradation existing in a liquefaction reaction. With a lower catalyst loading, degradation was favored and the LR percentage thus decreased rapidly. With a higher catalyst loading, the concentration of the liquefied products increased and re-polymerization was favored. Thus, the most appropriate catalyst loading was determined to be 3% for ROY liquefaction, which could provide a good balance between high liquefaction efficiency and effective retardation of re-polymerization of the liquefied products.

The liquefied product (PEG 400/ROY, 4.5/1; catalyst concentration, 3%; temperature, 160 °C; reaction time, 120 min) was diluted with methanol, and then filtered. The sample was silylated and then analyzed using GC-MS, and the identified compounds in the liquid are summarized in Table 2. Basically, the chemical components can be divided into six major groups: alcohols, acids, ketones, aldehydes, amides and esters. The alcohols include two main parts: liquefied products from degradation of polysaccharide, and PEG and its derivatives. The PEG derivatives included ethylene glycol (EG), triethylene glycol (TEG), tetraglycol and 15-Crown-5. Liquefaction of ROY using PEG produced a major compound of alcohol derivatives, which could be originating from degradation of polysaccharide forms acids and these acids further hydrolyzed polysaccharide to produce alcohol derivatives. Mannitol derivatives originating from decomposition of polysaccharide, such as dianhydromannitol, 5-hydroxy-3-oxopentanoic acid and (1S, 4S)-2,5-dioxabicyclo [2.2.2] octane-7,8-diol, were obtained in the final product. 2-[2-[2-(2-Hydroxyethoxy) ethoxy]ethoxy]ethyl acetate is formed from tetraglycol and acetic acid. Acetic acid is formed by cleavage of the acetyl group of sugar units. Furthermore, a small amount of fatty acids present in microbial oil was also detected in the liquefied product of ROY; these fatty acids included palmitic acid, stearic acid, (E)-octadec-9-enoic acid, (8E,11E)-octadeca-8,11-dienoic acid, and n-Hexadecanoic acid [21].

In general, ROY is mainly composed of polysaccharide, glucosamine and protein [3]. Thus, the mechanism of ROY liquefaction

Table 2. The chemical composition of liquefied product of ROY

No.	RT (min)	Compound name
1	6.848	n′-Formylformohydrazide
2	10.467	Thiazole
3	10.809	Ethylene glycol
4	11.299	4 h-imidazol-4-one
5	14.585	Ethanol
6	14.787	dl-Glyceraldehyde, dimethyl ether
7	16.18	Triethylene glycol
8	16.732	Caprolactam
9	16.873	Palmitic acid
10	17.230	(1s,4s)-2,5-Dioxabicyclo[2.2.2]octane-7,8-diol
11	17.241	Dianhydromannitol
12	17.924	Triethylene glycol
13	18.029	Pentaethylene glycol
14	18.362	15-Crown-5
15	18.52	Tetraglycol
16	18.573	5-Hydroxy-3-oxopentanoic acid
17	18.678	Stearic acid
18	18.871	(e)-Octadec-9-enoic acid
19	19.282	(8e,11e)-Octadeca-8,11-dienoic acid
20	19.615	1,4,7,10,13,16-Hexaoxacyclooctadecane
21	21.219	Tetraglycol
22	21.482	2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethyl acetate
23	21.862	15-Crown-5
24	22.201	1,4,7,10,13,16-Hexaoxacyclooctadecane
25	23.261	n-Hexadecanoic acid
26	23.813	15-Crown-5

in the presence of polyhydric alcohols may be as follows: polysaccharide is degraded and produces considerable EG-glucosides at the early stages of the liquefaction [22]. Then, the EG-glucosides are decomposed with the prolonging of liquefaction time, leading to large quantities of mannitol and its derivative. The compositional analysis of the residue from liquefaction is in progress.

CONCLUSIONS

The residue of oleaginous yeast (ROY) was liquefied in polyhydric alcohols using sulfuric acid as catalyst. The liquefaction conditions--liquefaction temperature, catalyst loading, reaction time, glycerol concentration and solvent/ROY ratio on the liquefied residue rate were evaluated. The results showed that the whole liquefaction process was divided into rapid liquefaction stage and slower liquefaction stage. The content of LR decreased with increase of liquor ratio. With increasing catalyst and react, the content of LR decreased to a minimum level and then abruptly increased with further increase of reaction time, catalyst loading, and liquefaction temperature, respectively. In contrast to lignocellulosic biomass liquefaction, glycerol had no effects on the retarded re-polymerization of liquefied products of ROY. The FT-IR results showed that the main component of ROY was polysaccharide. The GC-MS analyses showed that liquefied products of ROY included alcohols, acids, ketones, aldehydes, amides, esters and their derivatives.

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