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In situ self-catalyzed reactive extraction of germinated oilseed with short-chained dialkyl carbonates for biodiesel production

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HIGHLIGHTS

- Germinated seeds and DMC were used for in situ biodiesel production.
- The extra uses of catalyst and oil extraction solvent were avoided.
- The maximum biodiesel yield was 87.41% under the optimized conditions.
- The overall processing steps for biodiesel production can be reduced.

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ABSTRACT

In order to eliminate the expense associated with solvent extraction and oil cleanup, and reduce the processing steps in biodiesel production, reactive extraction has become a focus of research in recent years. In this study, germinated castor seed was used as substrate and catalyst, dimethyl carbonate (DMC) was used as acyl acceptor and oil extractant to produce biodiesel. The optimum conditions were as follows: the germination time of castor seed was 72 h, DMC/germinated seed ratio was 12.5 ml/g, reaction temperature was 35 °C, and water content was 2.11%. The biodiesel yield could reach as much as 87.41% under the optimized conditions. This germinated oilseed self-catalyzed reactive extraction can be a promising route for biodiesel production.

1. Introduction

Biodiesel, a renewable, biodegradable and non-toxic fuel, has emerged as one of the most potential candidates to replace current petro-diesel fuel (Shimada et al., 1999; Lam et al., 2010). Traditional methods for producing biodiesel from non-edible seeds oil involve various steps: oil extraction, purification, deacidification, dewaxing and a series of other processing, and subsequent esterification or transesterification. The requirement of these processing steps constitutes more than 70% of the total biodiesel production cost if refined oil is used as feedstock (Zeng et al., 2009; Shuit et al., 2010a). Recently, some researchers used "reactive extraction" technology for the production of biodiesel from oil-bearing materials, which can simplify the steps associated with solvent extraction and oil cleanup (Georgogianni et al., 2008; Qian et al., 2008; Ehimen et al., 2010; Kasim et al., 2010; Shuit et al., 2010b; Haas and Wagner, 2011a,b; Kiss and Bildea, 2012; Dong et al., 2013). Therefore, the reactive extraction is capable to replace traditional methods for biodiesel production.

According to the catalysts used in the reactive extraction process, the production methods of biodiesel can be broadly classified into two categories: chemical and enzymatic based transesterification (Su et al., 2009; Leung et al., 2010; El-Enin et al., 2013). Homogeneous acid or alkaline catalysts, such as H_2SO_4, NaOH, KOH and CH_3ONa, are mostly used in chemical reactive extraction thanks to the high catalytic efficiency, short reaction time and easy maneuverability. However, some disadvantages such as difficulties in products separation, equipments corrosion and wastewater pollution limit their applications (Leung et al., 2010). Enzymatic reactions can overcome these shortcomings, and have the advantages of low energy consumption, easy separation of products, mild reaction conditions and friendly to the environment (Su et al., 2009; Leung et al., 2010). However, the activity of lipase can be easily destroyed by the excessive use of short-chained alcohols (Shimada et al., 1999; Kumari et al., 2009). Su et al. have reported the production of biodiesel using dimethyl carbonate (DMC) as acyl acceptor, which could eliminate the risk of deactivation of lipase

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caused by short-chained alcohols (Su et al., 2007). Moreover, the reaction between oil and DMC is irreversible, and therefore enhance the reaction speed and improve the biodiesel yield (Su et al., 2007, 2009). However, the high cost and poor operational stability of the immobilized lipase become major bottlenecks of the enzymatic approach for biodiesel production (Shimada et al., 1999; Noureddini et al., 2005; Kumari et al., 2009). It is necessary to find a novel type of lipase for this enzyme-catalyzed reactive extraction technology. Recently, an environmentally friendly and low-cost in situ self-catalytic process for biodiesel production was developed (Gu et al., 2011). The highest fatty acid methyl ester (FAME) yield could reach 87.6% under the optimum conditions. Such a simple reactive extraction process without additional catalyst may greatly reduce the processing steps and costs of biodiesel production. In that work, *n*-hexane was used as co-solvent to accelerate the in situ transesterification. However, *n*-hexane was unfavorable to the lipase activity as well as to the separation of products. In order to avoid the extra use of extraction solvent and improve the stability of the lipase, DMC may be a better candidate, which can be used as acyl acceptor and extraction solvent at the same time.

Castor oil, extracted from the seeds of castor, is a non-edible plant oil source for biodiesel production, which ensures that biodiesel production does not compete with the food industry for resources (Ogunniyi, 2006; Berman et al., 2011; Hama and Kondo, 2013). Although there have been many studies reported on the production of biodiesel from castor seeds (Berman et al., 2011; Hincapié et al., 2011; Dias et al., 2013), there is no report on the in situ self-catalyzed process. Therefore, the main objective of the present work is to determine the feasibility of using reactive extraction without additional catalyst for the production of biodiesel from germinated castor seeds. DMC was used as acyl acceptor and extraction solvent. The optimal germination time of castor seed and reaction conditions (DMC dosage, reaction temperature, and water content) were investigated to obtain the maximum biodiesel yield.

2. Methods

2.1. Materials

The castor seeds (obtained from Fuao Seed Limited Company, Hebei Province, China) used in this study were standard products of Chinese agriculture. Anhydrous alcohol ($\geqslant$99.7% purity) and DMC were obtained from Tianjin Jiangtian chemical factory, Tianjin, China. Formaldehyde (37–40%) was obtained from Jinan Baiyun Chemical Limited Company, Jinan, China. All other chemicals and reagents were analytical pure and obtained commercially. All the organic solvents were treated with 4 Å molecular sieves for several days before use.

2.2. Preparation of germinated castor seed powder

Germinated castor seed powder was prepared according to the method of Gu et al. (2011). Castor seeds were soaked in 2% formaldehyde solution for 30 min to avoid fungal contamination and then washed several times with distilled water until the residual formaldehyde was removed thoroughly. Then, the disinfected seeds were soaked in tap water for 12 h to adequately absorb moisture. Thereafter, the seeds were placed in a culture dish paved with sheets of moist gauze, and were germinated at 27 °C in dark. Water was added at every 12 h to keep the gauze wetting. An amount of the germinated seeds were collected at every 24 h after seeding, and the water was removed through freeze-drying. Finally, the germinated seeds were milled to powder (particle size less than 1 mm) under 10 °C and stored at 4 °C.

2.3. Water and oil content of castor seeds

The castor kernels of different germination stages were milled to powder (particle size less than 1 mm) in advance and the obtained powder was used as the test sample.

Water content of castor seeds was measured gravimetrically using the official method of American Oil Chemists' Society (AOCS) Ai2-75 at 103 °C.

The theoretical oil content of castor seeds was determined according to AOCS Am2-93 using a soxhlet extractor with light petroleum as solvent. The amount of test sample and light petroleum was 4 g and 80 ml, respectively. The extraction process was carried out at 80 °C for 4 h. Light petroleum was removed by using a rotary evaporator after the extraction process and then the extracted oil was measured. The percentage yield was calculated on a dry weight basis.

2.4. Activity assay of the castor seed lipase

The germinated castor seed powder was washed with cold acetone for several times and the obtained white powder was named crude lipase. The hydrolytic activity of castor seed lipase was detected using an olive oil emulsion which contains 2% (w/v) polyvinyl alcohol as substrate. A mixture of 4 ml emulsion and 5 ml phosphate buffer (0.025 M, pH 5.0) was preheated at 37 °C for 10 min, and then a certain amount of the crude lipase was added into the mixture for another 30 min. The reaction was terminated through an addition of a mixture of ethanol and acetone (1:1, 15 ml) to inhibit the lipase activity (Kanwar et al., 2005). The quantity of the released fatty acid was measured by titration with 0.1 M KOH solution. One unit of lipase activity was defined as the amount of lipase that needed for olive oil to liberate 1 μmol of fatty acids per minute at 37 °C and pH 5.0.

2.5. Production of biodiesel

The oilseed powder (4 g) was mixed with a certain amount of DMC and the reaction system was kept tightly closed. The reaction was carried out at particular temperature with shaking speed of 180 r/min on a water bath shaker. Samples (200 μl) taken from the reaction mixture at specified times were analyzed quantitatively by gas chromatography (GC).

2.6. GC analysis of FAME yield

A gas chromatograph (SP-1000) which equipped with a hydrogen flame ionization detector (FID) was used in this study to detect the yield of FAME, and a capillary column (SE-30, 30 m × 0.25 mm) was employed for chromatographic analysis. The column temperature was kept at 160 °C for 2 min, and then heated to 200 °C at 15 °C/min. After that the heating process continued at 8 °C/min to 260 °C, with a holding time of 10 min. The temperature of the injector and detector were 240 and 280 °C, respectively. The yield of FAME can be calculated using the following equation:

$$\text{FAME yield}\ (\%) = \frac{(\sum \text{Concentration of each FAME}) \times (\text{Volume of upper layer})}{\text{Theoretical quantity of FAME}} \times 100\%$$

3. Results and discussion

3.1. Germination of the castor seeds

3.1.1. Morphological features of castor seed during different germination stages

Morphological features of castor seed during different germination stages were observed and the results were shown in Fig. S1. The imbibition or water uptake process is the fundamental requirement for the initiation and completion of seed germination. The testa of castor seed ruptured after 48 h imbibition, and hypocotyl elongation and radicle protrusion were observed until 72 h of imbibition. This process was termed as germination. As time went on, the hypocotyl and radicles continued elongation. Lipase activity and oil content were two key factors that determined the FAME yield. To obtain an overview of the changes of oil content and lipase activity during different germination stages, postgerminative growth until 144 h imbibition was included in this study.

3.1.2. Changes of oil content during different germination stages

The interconversion of oil and carbohydrate was known to occur in almost all germinating oilseeds including castor seed (Kornberg and Beevers, 1957). As shown in Fig. 1, the oil content of castor kernel was almost stable at germination stage. Compared to 64.00% at 0 h imbibition, the oil content was decreased slightly to 61.23% at 72 h imbibition. Obviously, utilization of the storage oil did not begin until this time. However, the oil content decreased sharply after 96 h imbibition and decreased to 20.66% after 144 h, indicating oil was mobilized and consumed during the postgermination (Yang et al., 2009). Actually, in the stage of postgermination, the seedling growth needed large amounts of energy and nutrition, which can be provided only by seed reserves such as oil, protein, and starch. In the endosperm of the castor seed, oil was the most abundant reserve (64.00%). Thus, the obvious declination of oil content was found after 96 h imbibition (Yang et al., 2009).

3.1.3. Changes of lipase activity during different germination stages

Lipase activity in most oilseeds could be detected during the germination stage, while for castor seed it could be detected even during the dormant state (Villeneuve, 2003). In oilseeds, lipase plays a fundamental role in oil mobilization (Staubmann et al., 1999). During the germination of castor seed, the primary stage in oil mobilization is commonly supposed to be the hydrolysis of triacylglycerol to glycerol and free fatty acids under the action of lipase (Rahman et al., 2008). The results given in Fig. 2 showed that the lipase activity in germinated seeds increased from 5.92 U/g (0 h) to a maximum of 29.25 U/g (72 h), and then the activity decreased to about 9.88 U/g (120 h). These results agreed with the decrease of oil content causing by catabolism after 72 h of germination (Fig. 1, Jachmanian et al., 1995). Based on the results of Figs. 1 and 2, it can be found that the activity of lipase in castor seed increased when oil was being utilized and declined as the oil was depleted, which were consistent with the previous report (Muto and Beevers, 1974).

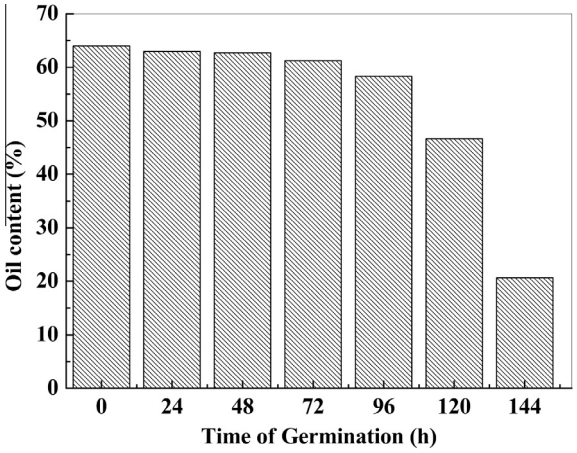

Fig. 1. Changes of oil content during different germination stages.

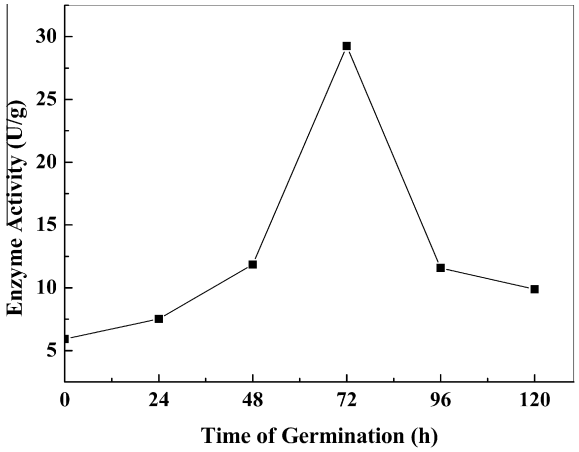

Fig. 2. Changes of lipase activity during different germination stages.

3.2. Effect of germination time on the yield of FAME

The FAME yield at different germination time was investigated and shown in Fig. 3. For a given germination time, the FAME yield increased gradually with the increase of reaction time, and the reaction went to an equilibrium after 8 h. Judging from different germination time, the FAME yield increased with the increase of germination time during the early stage of germination (0–72 h), and reached to a maximum of about 60.26% after seeding 72 h, which could be attributed to the high oil content and lipase activity (seeding 72 h). The FAME yield began to decrease when the germination time was further increased from 96 to 120 h. Based on the results of Figs. 1 and 2, these phenomena can be explained: the maximum lipase activity of the germinated seeds was detected at 72 h (Fig. 1). At that time, the germinated seeds featured only

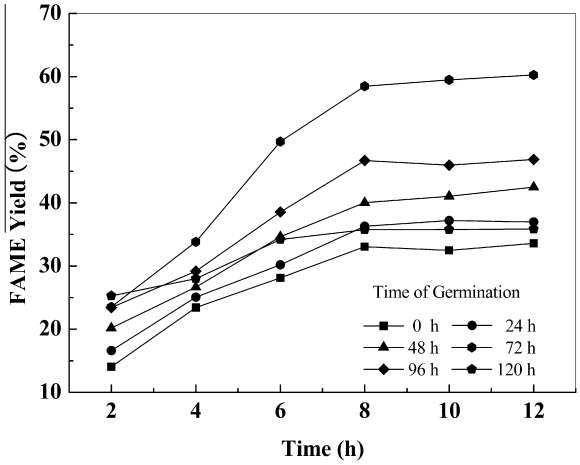

Fig. 3. Effect of germination time on the yield of FAME. Reaction conditions: 4 g of germinated seeds, 1.34% of water content, 10 ml/g of DMC dosage, 40 °C, 12 h, 180 r/min.

slightly lower oil content relative to the ungerminated seeds (Fig. 2). Therefore, the castor seeds that germinated for 72 h were used as the substrate for biodiesel production in the subsequent experiments.

3.3. Effect of DMC dosage on the yield of FAME

DMC dosage was one of the most important parameters in biodiesel production. Different DMC/germinated seed ratios (ml/g) were examined and the results were shown in Fig. 4. The highest FAME yield of 63.70% was obtained when the ratio was 12.5 ml/g. Shifting the ratio above or below the optimum ratio would cause a decrease of the FAME yield. This may be explained as follows: DMC acted as extraction solvent and acyl acceptor in the reaction–extraction process, which had no impair on the activity of lipase, but it could produce substrate inhibition on transesterification (Su et al., 2009). When a low DMC/germinated seed ratio was used, the solvent was not enough to thoroughly extract the oil (Su et al., 2007, 2009); when the ratio was high, the over used DMC could lead to an excessive dilution of oil and substrate inhibition (Su et al., 2009). Additionally, the diffusion speed of DMC into the oilseed powders also influenced the reaction rate of in situ transesterification. A suitable DMC dosage would be required to overcome substantial mass transfer resistance in order for the reaction to proceed at an appreciable rate (Kasim et al., 2010). Thus, the optimum DMC/germinated seed ratio of 12.5 ml/g was adopted in the subsequent experiments.

3.4. Effect of reaction temperature on the yield of FAME

Experiments were performed over the temperature range of 30–45 °C to examine the effect of reaction temperature on the FAME yield, and the results were shown in Fig. 5. Generally, a higher reaction temperature would lead to a higher reaction rate, which corresponded to a higher biodiesel yield (Su et al., 2007; Goembira and Saka, 2013). However, enzymes were sensitive with temperature and easily deactivated at high temperature (Su et al., 2007). In this work, FAME yield was found to continuously increase with the increase of reaction temperature, until a highest yield of 84.45% was obtained at 35 °C, which was resulted from the increased lipase activity that accelerated the reaction. When the reaction temperature was kept above 35 °C, the FAME yield decreased. This was resulted from the following reasons: firstly, high temperature could lead to the deactivation of lipase, which was unfavorable to the reaction; Secondly, the increased reaction temperature favored the hydrolytic activity of lipase, which was undesired for the biodiesel production (Noureddini et al., 2005). Thus, the optimum reaction temperature was 35 °C and it was adopted in the subsequent experiments.

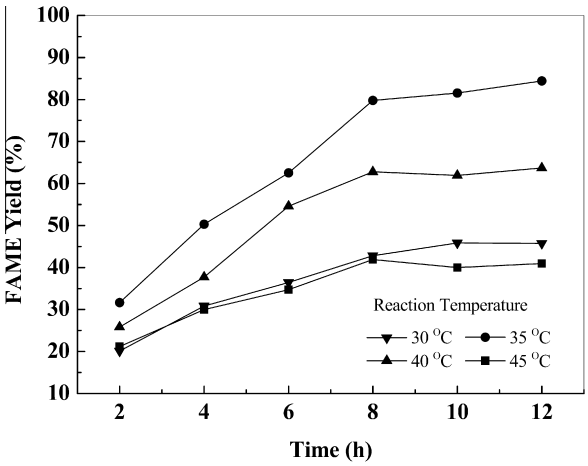

Fig. 5. Effect of reaction temperature on the yield of FAME. Reaction conditions: 4 g of germinated seeds, 1.34% of water content, 72 h of germination time, 12.5 ml/g of DMC dosage, 12 h, 180 r/min.

3.5. Effect of water content on the yield of FAME

Enzymatic biodiesel production is usually conducted in non-aqueous media. In this process, water plays important roles and has strong influence on the activity and stability of lipase (Lu et al., 2009). Thus, some essential water is required for lipase to exhibit biological activity through the formation of oil–water interface (Noureddini et al., 2005; Su et al., 2007; Lu et al., 2009). However, because the reaction is reversible, when water content beyond the optimum value, hydrolytic reactions catalyzed by lipase become significant and the FAME yield is supposed to go down (Noureddini et al., 2005; Su et al., 2007, 2009; Lu et al., 2009). Thus, the optimum water content is a compromise between minimizing hydrolysis and maximizing enzyme activity for the transesterification reaction (Noureddini et al., 2005; Su et al., 2007). As can be seen in Fig. 6, the highest FAME yield (87.41%) was attained at 2.11% of water content. When the water content

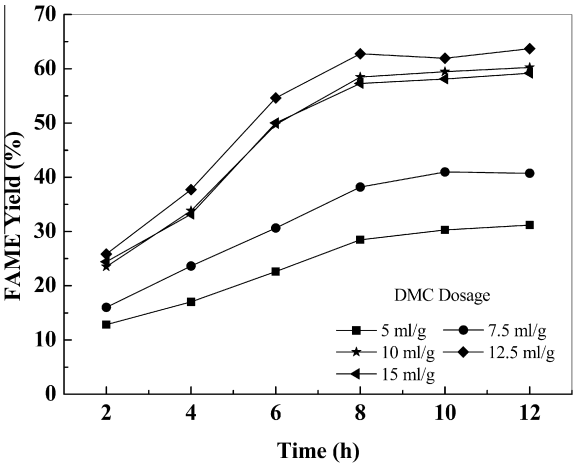

Fig. 4. Effect of DMC dosage on the yield of FAME. Reaction conditions: 4 g of germinated seeds, 1.34% of water content, 72 h of germination time, 40 °C, 12 h, 180 r/min.

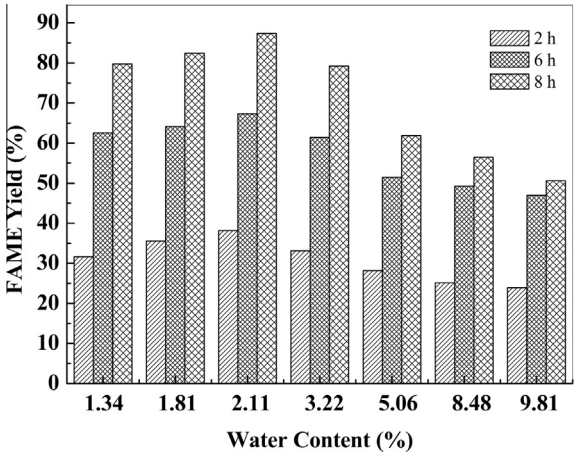

Fig. 6. Effect of water content on the yield of FAME. Reaction conditions: 4 g of germinated seeds, 72 h of germination time, 12.5 ml/g of DMC dosage, 8 h, 35 °C, 180 r/min.

deviated from the optimum value, the FAME yield decreased. Thus, the water content of 2.11% was adopted for biodiesel production. Analysis of the biodiesel produced by this method indicated that the quality of the biodiesel meets all specifications of the American Society for Testing Materials (ASTM) and the China Criteria (data not shown).

4. Conclusions

Germinated castor seeds and DMC were used to produce biodiesel, and the maximum biodiesel yield was 87.41% under the optimized conditions. This method reduces the overall biodiesel processing steps through combining the extraction and transesterification processes in one step. Furthermore, this method that avoids additional lipase and extractant added will cut all the cost associated with the catalyst and co-solvent and simplify the downstream processes. Although there may be many challenges remaining before commercializing this technology, the in situ self-catalyzed reactive extraction of germinated oilseed with short-chained dialkyl carbonates probably will become a promising method for biodiesel production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.09.127.

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