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Co-production of biodiesel and methacrylated fatty acid through enzymatic catalysis with methyl methacrylate as acyl acceptor

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ABSTRACT

Traditional biodiesel production primarily uses methanol as the acyl acceptor, but its toxicity to lipase increases process complexity and operational difficulty elevate manufacturing costs. This study aimed to explore a new method for enzymatic synthesis of biodiesel with methyl methacrylate (MMA) as acyl acceptor. Meanwhile, a 1, 3-position specific lipase Lipozyme RM IM was applied as biocatalyst, which enables simultaneous production of biodiesel (FAMES) and methacrylate fatty acid glycerides (MFAGs) via specific *sn*-1, 3 transesterification of MMA with triglyceride. Under the optimal reaction conditions: temperature of 50 °C, molar ratio of 4:1 for MMA to triglyceride, enzyme dosage of 7.5% (mass), and an extra water addition of 0.5% (mass); triglyceride conversion rate of 97%, and FAMES yield of 65% could be obtained. Simultaneously, the multistage short-path distillation and column chromatographic method were combined used for the separation of the mixed products. Finally, the purity of FAME, MFADG, DMFAG, and MMFAG were 98%, 97.8%, 95.3%, and 81.78%, respectively. In this new approach, MMA demonstrates lower toxicity to lipases, allowing for straightforward addition of all the substrates without complex addition process, and enhancing operational feasibility. Meanwhile, the by-products of MFAGs could be applied as monomers in varnishes and protective coatings, which increased the value of the products. Thus, this investigation providing an alternative way to produce biodiesel, and providing a new pathway for the sustainable development of biodiesel.

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1. Introduction

Biodiesel, a well-known biofuel, is made from the renewable biological resources and can be applied as a substitution of fossil fuel. Its production capacity in 2020 represented 15% of the total diesel production [1]. It has been utilized for transportation, shipping, and agriculture [2]. Due to the exhaustion of petroleum, and the increasingly serious problem of environmental pollution, biodiesel production technologies have been developed rapidly in the last decade [3]. Normally, biodiesel refers to a mixture of free fatty acid (FFA) alkyl ester, produced through the transesterification of oils or fats with an acyl acceptor.

Traditional biodiesel production typically employs strong acid or base catalysts such as H₂SO₄ or NaOH, which, although effective in promoting transesterification. However, the chemical catalysis requires harsh reaction conditions, non-specific substrate interactions leading to byproduct formation, and environmental impact from toxic reagents. Compared to chemical catalysis, enzymatic catalysis would enhance substrate specificity, with operation under ambient conditions (25–40 °C, atmospheric pressure), and environmentally friendly. Even though the enzymatic method has the advantages listed above, the unstable property of the lipases, and the high cost of the process limited the industrial application of the enzymatic method [4,5].

Traditionally, the acyl acceptor applied for biodiesel is methanol, so the biodiesel was commonly recognized as fatty acid methyl esters (FAMES). The application of methanol was mainly because of the lower price than other alcohols. However, methanol may disrupt the hydrophobic interactions and hydrogen bonding network within enzyme molecules, leading to alterations in enzyme conformation [6,7]. This process involves solvent-induced

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destabilization of the enzyme's tertiary and secondary structures, which are critical for maintaining catalytic activity [8,9]. For enzymatic biodiesel production, the reversibility of transesterification necessitates excess alcohol to drive equilibrium toward fatty acid methyl ester (FAME) formation. While substrate molar ratios of methanol to oil between 3:1 and 6:1 are commonly employed [10,11], experimental evidence indicates that methanol additions exceeding 1:1 (vol) induce lipase denaturation, simultaneously reducing biodiesel yield and enzyme reusability [12,13]. To mitigate methanol toxicity, stepwise methanol addition has proven to be the most effective strategy [14], and was the most regularly applied strategies to reduce the impact. However, stepwise controlled feeding approach increases process complexity, operational difficulty, equipment requirements, and overall production time, thereby elevating manufacturing costs [15,16]. The choice of an alternative acyl acceptor with lower toxicity to the lipase was another approach to solve the problem. Compared to methanol, ethanol or butanol were more friendly to the lipase, but the higher price of those alcohols must be considered. Since the lipase has the ability to catalyze transesterification between two esters, technologies with methyl acetate [17,18] or ethyl acetate [19] as the acyl acceptor were also investigated. Due to the nonpolar microenvironment, and without glycerol produced, the conversion rate and the life of lipase were greatly improved [20]. In addition, the process would be economically viable, because of the value-added by-product of triacetin, which has been widely applied as food additive, emulsifier, plasticizer [21,22], and stabilizer of fuels [23]. Except for acetate, dimethyl carbonate was also utilized as an alternative to methanol, and a value-added by-product glycerol carbonate was obtained [24]. The advantages of organic acid esters as acyl acceptors demonstrated that a suitable ester has the potential for the alternative of short-chain alcohols.

Methyl methacrylate (MMA) is a crucial chemical raw material, which could be applied as the monomer for many kinds of polymers [25]. Even though there are lots of manufacturing methods, the dominant commercial process reacts acetone and hydrogen cyanide to MMA directly (ACH route) [26]. Thus, the commercial price of MMA is usually lower than methacrylic acid (MAA). When MMA (Compound 1) is applied as acyl acceptor for the transesterification with plant oil (triglycerides, TAGs, Compound 2) (Scheme 1), by-products of methacrylate fatty acid diglyceride (MFADG, Compound 6), mono-methacrylate fatty acid glyceride (MMFAG, Compound 3), and di-methacrylate fatty acid glyceride (DMFAG, Compound 5), would be obtained apart from biodiesel (FAMEs, Compound 4). Methacrylate fatty acid glycerides (MFAGs, include MMFAG, DMFAG, and MFADG) could be utilized as materials for the manufacture of paints, varnishes, printing inks, and protective coatings through copolymerization with vinyl monomers [27]. Meanwhile, because MFAGs and its derivatives have significantly lower HAPs/VOCs (hazardous air pollutants/volatile organic compounds) emissions than e.g. styrene, they were utilized reactive diluents to replace or reduce the dosage of styrene in the manufacture of vinyl ester (VE) and unsaturated polyester (UPE) resins [28–30]. Recently, it was found that polymers with MFAGs derivatives as precursors exhibited thermoregulated shape memory properties, which indicated that those compounds have the potential for special polymer manufacture in the future [31,32].

Normally, MFAG was synthesized from the addition reaction with the epoxide group of glycidyl methacrylate (GMA) by the carboxylic group of fatty acids [31,33]. Compared to the conventional process, utilizing MMA as an acyl acceptor could simultaneously produce biodiesel and biomaterial precursors, which may reduce the influence of short-chain alcohol on lipase, and affect positively the economics of the biodiesel production process.

As illustrated in Scheme 1, a *sn*-1,3-regioselective catalyst is essential for the simultaneous production of biodiesel (FAMEs) and MFAGs via transesterification of MMA with triglycerides. Lipozyme RM IM, an immobilized lipase from *Rhizomucor miehei*, demonstrates exceptional 1,3-regioselectivity would be a suitable biocatalyst for this integrated synthesis process. Thus, in this work, the coproduction process of biodiesel and MFAGs through MMA as an acyl acceptor was investigated. Lipase of Lipozyme RM IM was introduced as a biocatalyst, and the enzymatic multiple transesterification process was optimized. Finally, the separation of MFAGs from biodiesel was also studied. The described concept provides an alternative way for biodiesel production.

2. Materials and Methods

2.1. Chemicals

Glycerol trioleate (TG) is purchased from Shanghai Yuanye Bio-Tech Co. Ltd (China), and methyl methacrylate (MMA, with 0.003% MEHQ) is purchased from Macklin Co. Ltd (China). Lipozyme RM IM (lipase from *Rhizomucor miehei*, initial enzyme activity was 220 IU·g⁻¹) is purchased from Novozymes (Denmark). Other reagents are analytical reagent and purchased from Beijing Chemical Works (China).

2.2. Enzyme activity assay

The enzyme activity was monitored before and after each batch of reaction. After the reaction, the immobilized lipase was separated through filtration (with a 0.45 μ m Nylon filtration membrane), and then washed with 10 ml *n*-hexane. Finally, the enzyme activity was assayed by the method of olive oil hydrolysis.

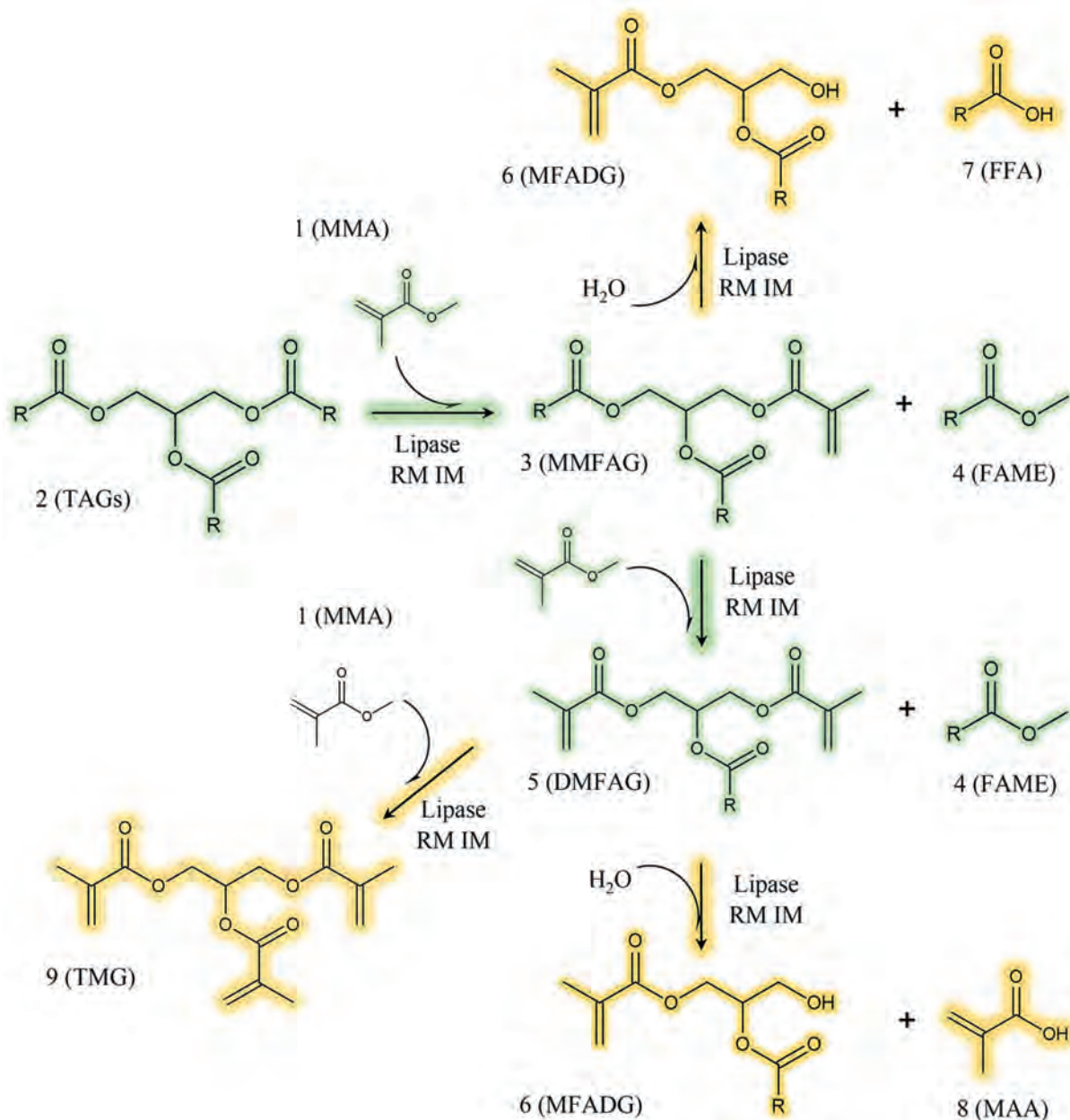
The details for the enzyme activity assay were as follow: the assay system comprised of 5% (vol) olive oil emulsified in distilled water containing 2% polyvinyl alcohol, 4 mL phosphate buffer (100 mmol·L⁻¹, pH 8.0), and 10 mg immobilized enzyme. The hydrolysis reaction was performed under constant agitation at 35 °C for 10 min. Then, the reaction was terminated by 20 ml ethanol addition. The released fatty acids were quantified through titration with 50 mmol·L⁻¹ NaOH. One unit (IU) of enzyme activity was defined as the amount of enzyme that released 1 mmol fatty acid per minute under assay conditions.

2.3. General procedure for the transesterification process

Unless otherwise specified, the transesterification process was performed in a solvent-free form. The Lipozyme RM IM catalyzed transesterification was carried out in a 5 ml glass bottle, the foundational parameters before optimization were set as follow: the molar ratio of MMA to the TG was 4:1, with lipase loading of 7.5% (enzyme: TG, mass) and water addition of 0.5%. For the reaction condition optimization, the temperature was determined at 40, 45, 50, 55 and 60 °C, the water loading was determined at 0%, 0.5%, 1%, 1.5%, and 2%, the enzyme loading was determined at 5%, 7.5%, 10%, and 12.5% (enzyme: TG), and the molar ratio of MMA to the TG was determined at 2:1 to 6:1. For the analysis, 10 μ l sample was taken, and 1 ml *n*-hexane was added as solvent for the following GC analysis.

2.4. Recovery and batch use of enzymes

After each catalytic batch operation, the immobilized enzyme was separated from reaction liquid (vacuum filtration with a 0.45 μ m Nylon filtration membrane), and then subjected to an oscillatory extraction with *n*-hexane to effectively extract residual organic reactants and byproducts. Subsequently, the enzyme was



Scheme 1. The multiple transesterification process of TAGs with MMA, main and side reaction. The main reaction pathway was in green, and the side-reaction was in yellow.

dried under ambient conditions with vacuum-assisted desiccation ($25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, $15\text{--}20\text{ kPa}$). Then, according to the general procedure for the transesterification process, the reusability of the immobilized enzyme was tested.

2.5. The separation of the products

The scaled-up system was conducted in a 2 L agitated reactor at $400\text{ r}\cdot\text{min}^{-1}$, $50\text{ }^{\circ}\text{C}$ for 24 h. At the end of the reaction, the enzyme powder is removed by vacuum filtration (with $0.45\text{ }\mu\text{m}$ Nylon filtration membrane). Separation of multiple substances was done by molecular distillation (VTA GmbH & Co.KG) based on differences in the mean free path of molecules between multiple products. The supernatant was collected and separated by short-path distillation (SPD) with the following conditions, distillation 1: $T_{\text{Evaporation wall}} = 135\text{ }^{\circ}\text{C}$, $T_{\text{Cooling wall}} = 5\text{ }^{\circ}\text{C}$, scraper speed = $185\text{ r}\cdot\text{min}^{-1}$, and pressure = 0.1 Pa . FAME was obtained as the light phase. And then

the secondary distillation was performed to further enhance the purity of FAME, distillation 2: $T_{\text{Evaporation wall}} = 60\text{ }^{\circ}\text{C}$, $T_{\text{Cooling wall}} = 5\text{ }^{\circ}\text{C}$, scraper speed = $230\text{ r}\cdot\text{min}^{-1}$, and pressure = 0.1 Pa . The heavy phase from distillation 1 was separated by distillation 3: $T_{\text{Evaporation wall}} = 165\text{ }^{\circ}\text{C}$, $T_{\text{Cooling wall}} = 20\text{ }^{\circ}\text{C}$, scraper speed = $230\text{ r}\cdot\text{min}^{-1}$, and pressure = 0.1 Pa . Under this condition, DMFAG and MMFAG were separated, with DMFAG in the light phase and MMFAG in the heavy one.

2.6. The separation of DMFAG and MFADG

For the further separation of DMFAG and MFADG, the light phase from distillation 3 was further purified by silica gel column chromatography. Approximately 350 g of silica gel (200–300 mesh, $0.049\text{--}0.074\text{ }\mu\text{m}$) was packed into a glass column (457 mm length $\times$ 80 mm i.d. , XINWEIER GLASS Co., Ltd., China). The mixture was eluted using a mobile phase of *n*-hexane and ethyl acetate (80:

20, vol). Fractions were collected at regular intervals and analyzed by gas chromatography to monitor component separation. The collected fractions containing DMFAG or MFADG were concentrated through vacuum evaporation to remove the solvent, affording purified DMFAG and MFADG. The final purity of DMFAG and MFADG was analyzed by GC.

2.7. Analytical procedure

The esterification mixture was analyzed on a Shimadzu GC-2010 (Shimadzu, Japan) equipped with a DB-1ht column (30 m × 0.25 mm × 0.1 μm, Agilent technologies) with nitrogen as carrier gas. The detector temperature was 380 °C and the initial column temperature was set at 60 °C with a rate of increase to 375 °C at 9 °C·min⁻¹ and held for 15 min.

The FAME yield was quantified through the following equation. Where N_{FAME} was the molar quantity of FAME produced during the reaction. M_{TG} was the initial molar quantity of substrate TG add into the reaction.

$$\text{FAME yield} = \frac{N_{\text{FAME}}}{M_{\text{TG}} \times 3} \times 100\%$$

2.8. Properties assay

The weighing method was applied for liquid density determination. Kinematic viscosity at 40 °C was analyzed by a SYD-265H kinematic viscosity tester (Shanghai Changji Geological Instrument Co., Ltd.). Thermogravimetric analysis was carried out with PerkinElmer thermogravimetric differential thermal analyzer (TG/DTA). The flow rate of nitrogen was 40 ml·min⁻¹, the heat rate was set at 10 °C·min⁻¹, and the heating temperature ranged from 30 °C to 600 °C.

Acid value was tested with a titration method. Sample was weighed (accurate to the 0.001 g) and dissolved in 50 ml 95% ethanol. The solution was titrated with a 0.1 mol·L⁻¹ KOH solution. The titration was with thymol blue as indicator and stopped with thymol blue titrates to a blue-green color. The acid value of the sample, expressed as milligrams of KOH per gram of sample, which was calculated as follows:

$$\text{Acid number} = (56.11 \times A \times N) / B$$

Where, A was the volume of alkali solution consumed in titration of the sample, ml; N was the normality of the alkali solution; B was the mass of specimen, g.

2.9. Statistical analysis

The experiments were conducted in triplicate and the results were presented as the mean ± standard deviation (SD). GraphPad Prism 9.5 software (Dotmatics, Boston, MA, USA) was used to generate charts.

3. Results and Discussion

3.1. Influence of water loading of the reaction

The aim of this work is co-production of MFAGs and FAMES from the transesterification of MMA and triglyceride. Most lipases exhibit good transesterification activity. Suitable enzymes for this process were first selected. Commercial immobilized lipase Novozym 435 was the most commonly applied biocatalyst for biodiesel production, and with the excellent catalytic performance of the enzyme. In this research, Novozym 435 was choose as control to check the necessary of a position specific lipase to this aim of the investigation. As the results of control experiments, a high FAME yield of 95% could be obtained in this process. However, since the weak regioselectivity of the enzyme, all of three fatty acids on the glycerol skeleton were entirely transformed into FAMES, without the by-products of MFAGs. Thus, a *sn*-1,3 specific lipase was necessary for the aim of this investigation, which could catalyze the transesterifications between MMA and triglyceride only in the position *sn*-1 or *sn*-3 of glyceryl. As a remarkable *sn*-1,3 specific lipase Lipozyme RM IM was chosen as catalyst for the following investigation in this work.

With the catalytic of Lipozyme RM IM, the main products of the transesterifications are MMFAGs, DMFAGs, and FAMES. From Fig. 1(a), it could be found that the conversion rate of TGs and the yield of FAMES was increased with the increase of water loading within the range of the experiment. The water is necessary for the reaction. A critical water content is necessary for maintaining the active configuration of the enzyme, but increased water loading would enhance the emulsification of the reaction system, and hence accelerate the enzymatic reaction rate [34,35]. However, pronounced side-reaction of

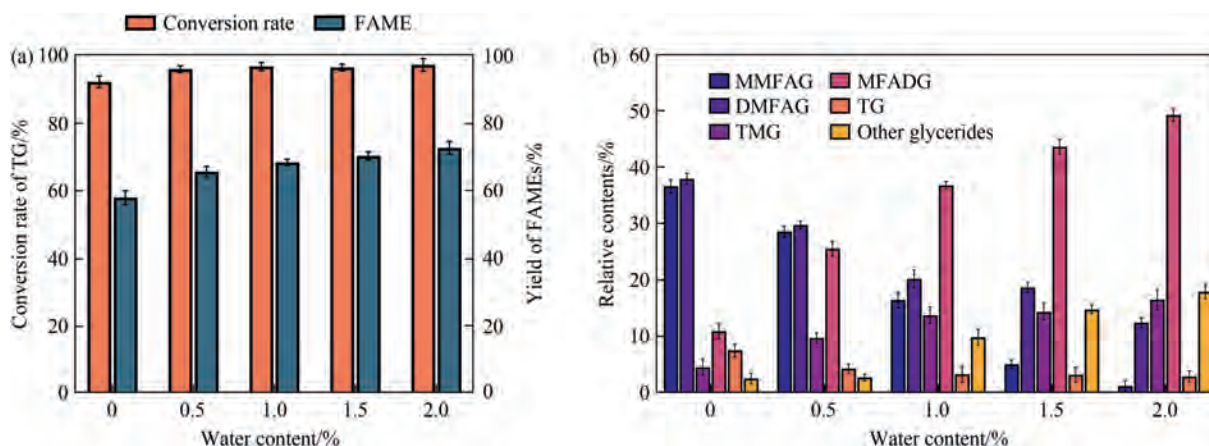


Fig. 1. Influence of water loading for the reaction. Reaction conditions: molar ratio of MMA to the TG was 4:1, with lipase loading of 7.5% (enzyme: TG, mass), water added 0% to 2% and reaction temperature at 50 °C; (a) the conversion rate of TGs and yield of FAMES in different water content. (b) The relative content of products in different water content. MMFAG: mono-methacrylated fatty acid glyceride; DMFAG: di-methacrylated fatty acid glyceride; TMG: glycerol tri-methacrylate; MFADG: methacrylated fatty acid; TG: glycerol trioleate; other glyceride: mono-, di-glycerides, and glycerol mono-/di-methacrylate; FAME: free fatty acid methyl esters, biodiesel.

hydrolysis would take place with water, and unfavorable by-products will be accumulated. As illustrated in Fig. 1(b), the decrease in MMFAG and DMFAG contents with increased water loading was generally attributed to hydrolysis. Particularly, as one by-product of the hydrolysis, MFAs exhibited excellent performance when applied as reactive diluents for copolymerization with vinyl monomers [28–30]. Hence, when MFAs were recognized as a part of target products, the appropriate water content is necessary. It should be noticed that increasing the water loading would increase the yields of MFAs (Fig. 1), but much hydrolysis will take place when the water loading is above 0.5%, reflected in decreasing MMFAGs and DMFAGs contents and accumulation of by-products of other glycerides. The main contents of other glycerides were mono-/di-glycerides, and glycerol mono-/di-methacrylate, which were difficult to separate from the main products since the similar boiling points of those glycerides. Therefore, the water loading of 0.5% was chosen for the subsequent investigation.

3.2. Catalyst dosage

The enzyme loading will influence the reaction efficiency and the cost of the enzymatic process. The appropriate amount of enzyme addition ensures increased substrate binding site availability, thereby facilitate the enzymatic reaction. Under excess reactant conditions and mass-transfer-unlimited systems, increasing catalyst loading significantly improves reaction rate. However, as the enzyme cannot shift the reaction equilibrium, continue increasing enzyme loading above the optimal dosage could not further enhance conversion efficiency but raises operational costs.

In this investigation, the influence of catalyst dosages was examined by varying lipase usage from 5% to 12.5% (based on the mass of TGs). As illustrated in Fig. 2(a), the yields of FAMES remained stable when the lipase dosage was higher than 7.5%. At the same time, for the products MMFAG, DMFAG, and MFA, their yields remained stable with increasing amounts of enzyme, with only small variations, even greater amounts of enzyme may affect the mass transfer of the system, considering the cost of the process, the optimum catalyst dosage was chosen at 7.5%.

3.3. Substrate ratio

Due to the difference in viscosity between the two substrates, it could be found that the system viscosity decreases with an

increase in the ratio of substrates (MMA to TG), which in turn favors an increase in mass transfer. At the same time. The molar ratio of the substrate directly affects the yields of the various products. Thus, controlling the ratio of substrates is extremely valuable in this pathway. More MMA could replace more fatty acid molecules in the transesterification, and as the ratio of MMA to TG increased, the conversion of TG increased significantly, and correspondingly more FAMES were obtained (Fig. 3(a)). Changes in the ratio of substrates would provide more opportunities for transesterification, and would also affect the equilibrium point of the reaction, the relative content of DMFAG was greatest at a molar ratio of 4:1 (MMA: TG) and the relative content of each major by-product stabilized as it increased further (Fig. 3(b)). This ratio was considered to be the optimum substrate ratio for this process.

3.4. Reaction temperature

Temperature is a frequently discussed parameter in the application of lipases [36–38] and it has a great influence on the efficiency of the catalysis. The optimum working temperature for lipases normally occurs between 40 °C and 60 °C, and lipases of microbial origin have a higher thermal resistance. An appropriate increase in the temperature of the transesterification system can effectively promote acyl transfer to obtain more of the target product. However, due to the nature of lipase as a protein, higher temperatures can lead to denaturation and thus affect catalytic activity [39]. As shown in Fig. 4, the conversion of TG stabilized at around 97% after the temperature reached 45 °C and the highest DMFAG relative content occurred at 50 °C. The reaction temperature of 50 °C is considered to be the optimum for this system in terms of economic efficiency.

3.5. Transesterification under the optimal conditions

As shown in Fig. 5, changes of substrates and products were recorded after 72 h under the optimal production conditions. The yield of FAME, the direct product of acyl transfer, showed the same pattern of variation as the conversion of TG and was stable at 65% after 10 h. This is consistent with the catalytic properties of 1,3-specific lipase, while the longer time for the reaction to reach equilibrium may be related to the effect of the viscosity of the system on mass transfer. MMFAG showed a rapid increase at the beginning of the reaction and further collisions would bring about more acyl transfer, while the relative amount of DMFAG began to dominate. The catalytic specificity of the *sn*-1,3 position of RM IM

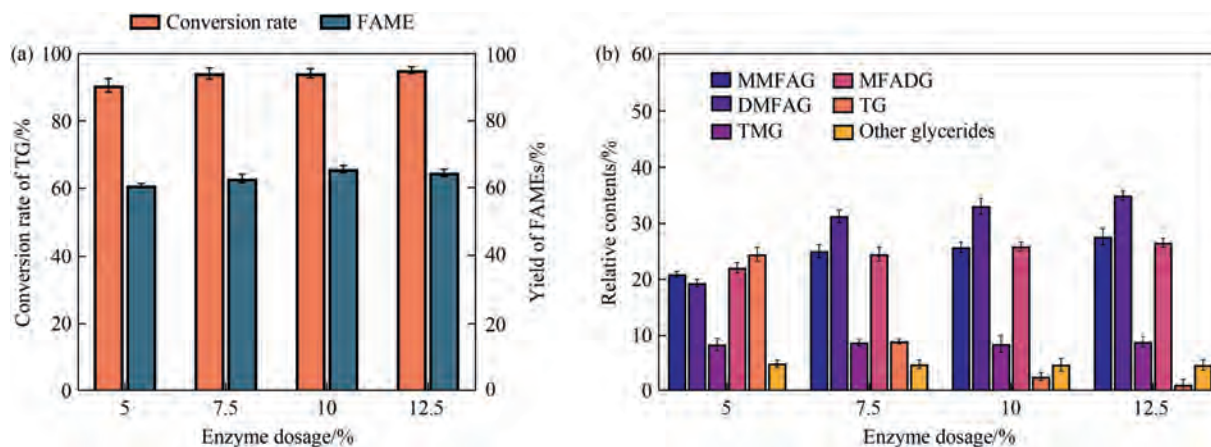


Fig. 2. Influence of enzyme loading for the reaction. Reaction conditions: the molar ratio of MMA to the TG was 4:1, with lipase loading of 5% to 12.5% (enzyme: TG, mass), water added of 0.5% and reaction temperature at 50 °C. (a) The conversion rate of TGs and yield of FAMES in different enzyme dosage. (b) The relative content of products in different enzyme dosage.

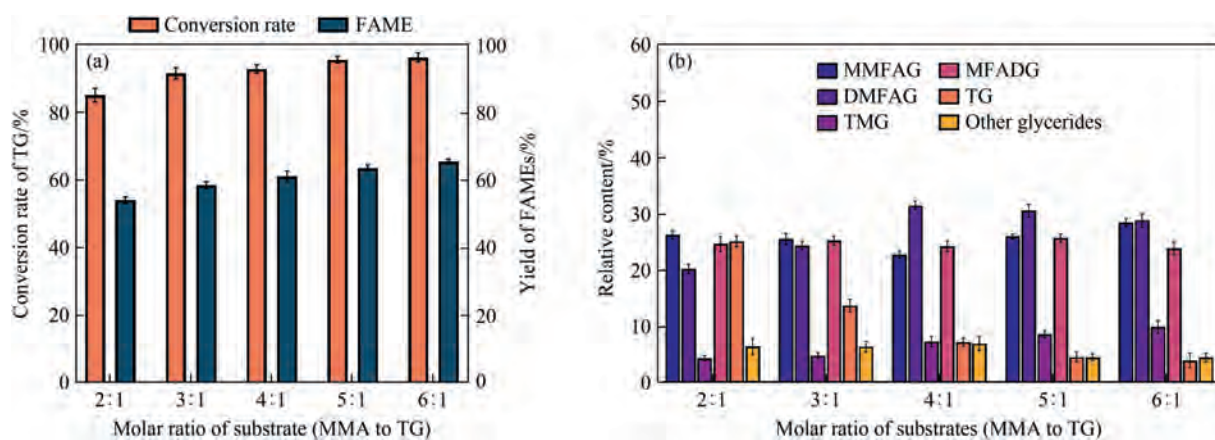


Fig. 3. Influence of molar ratio of substrate for the reaction. Reaction conditions: the molar ratio of MMA to the TG was 2:1 to 6:1, with lipase loading of 7.5% (enzyme: TG, mass), water added of 0.5% and reaction temperature at 50 °C. (a) The conversion rate of TG and yield of FAMES in different molar ratio of substrate. (b) The relative content of products in different molar ratio of substrate.

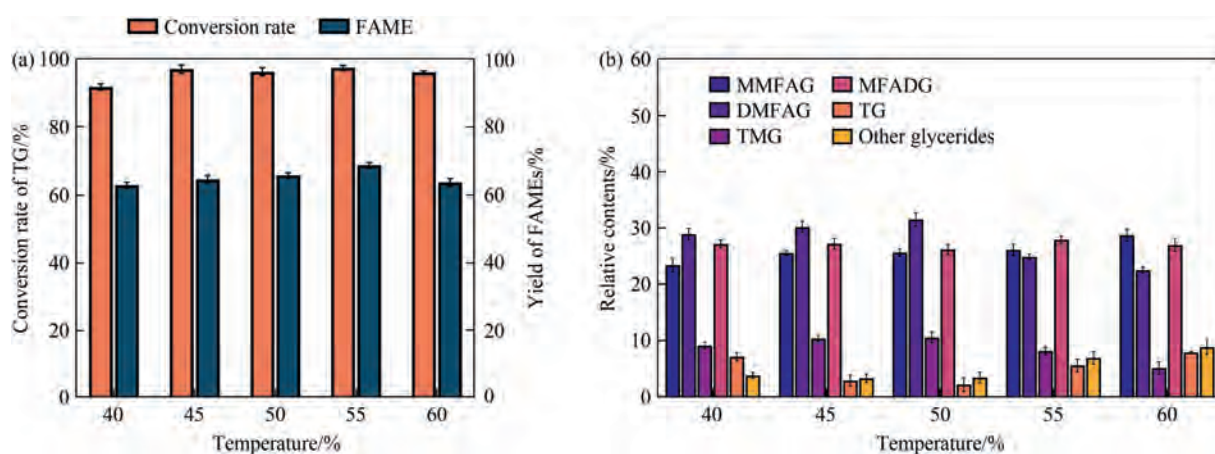


Fig. 4. Influence of temperature for the reaction. Reaction conditions: the molar ratio of MMA to the TG was 4:1, with lipase loading of 7.5% (enzyme: TG, mass), water added of 0.5% and reaction temperature at 40 °C to 60 °C. (a) The conversion rate of TG and yield of FAMES in different temperature. (b) The relative content of products in different temperature.

lipase greatly limited the occurrence of the *sn*-2 exchange of TG and the addition of water promoted the migration of TG, allowing a yield of about 8% of TMG to be achieved.

The reusability of the immobilized enzyme constitutes a critical parameter in assessing the economic viability of the enzymatic process. The decline in biocatalyst activity following batch reactions may from two primary factors: (1) structural denaturation

of lipase induced by repeated thermal or solvent stress, and (2) progressive accumulation of impurity on the immobilized enzyme matrix, which creates diffusion limitations and active site blockage.

To investigate the impact of impurity accumulation during repeated reaction cycles, regeneration protocols incorporating solvent washing steps were implemented in comparative experiments. It was found that solvent washing treatment demonstrated

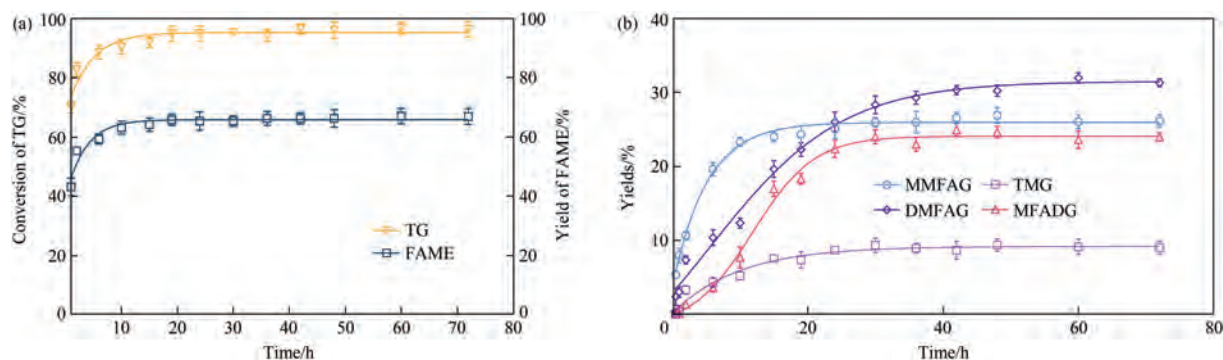


Fig. 5. Transesterification under the optimal conditions. Reaction conditions: the molar ratio of MMA to the TG was 4:1, with lipase loading of 7.5% (enzyme: TG, mass), water added of 0.5% and reaction temperature at 50 °C. (a) The conversion rate of TG and yield of FAMES in the optimal conditions. (b) The relative content of products in different temperature. Lines in the figures were the trend line from experimental datasets.

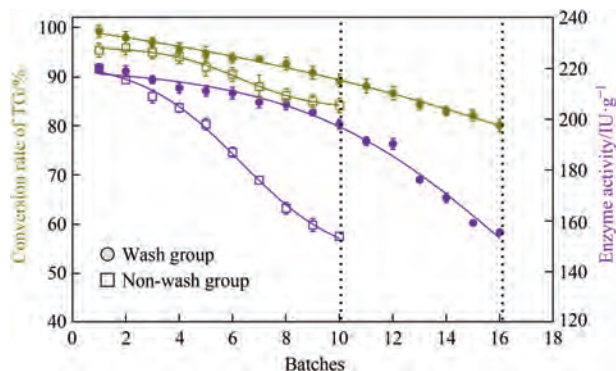


Fig. 6. Conversion of TG by different batches of RM IM lipase.

enhanced operational reusability of the immobilized enzyme (Fig. 6). The 20% activity loss (from 220 IU/g to 175 IU/g) from 10 cycles without solvent washing changed to 16 cycles with the treatment of washing. These findings confirmed that solvent washing effectively preserved enzyme activity, thereby significantly enhancing operational stability. Solvent-washing treatment

enabled the immobilized lipase to achieve 16 catalytic cycles with a 19% conversion efficiency decline (from 99% to 80%). Moreover, Using MMA as the substrate significantly enhanced lipase reusability compared to methanol, underscoring methanol's limitations on enzyme catalytic performance during repeated batch operations (Table 1). This performance validated the feasibility of the transesterification process utilizing TG and MMA for biodiesel production and high-value co-products synthesis.

3.6. The separation of products

The separation of the reaction mixture was achieved through short-path distillation (SPD), a highly efficient technique particularly suitable for isolation of glyceride mixtures with high boiling point. Since the components of the reaction mixture were complex, three times of short-path distillation and silica gel-based column chromatography were applied to make each product separated (Fig. 7). In the first time of SPD (SPD-1, 0.1 Pa, $T_{EW} = 135\text{ }^{\circ}\text{C}$, $T_{CW} = 5\text{ }^{\circ}\text{C}$), FAME was separated from the mixture of glycerides, and then further purified with second time of SPD (SPD-2, 0.1 Pa, $T_{EW} = 60\text{ }^{\circ}\text{C}$, $T_{CW} = 5\text{ }^{\circ}\text{C}$), with a purity increased from 96.41% to 98.00%. The mixed glycerides (mixture of MMFAG, DMFAG, MFADG, and TG) were concentrated in the heavy phase of SPD-1, and the

Table 1

Comparison of lipase reusability using methanol and MMA as substrates.

Lipase	Reutilization	Method	Ref.
<i>C. antarctica</i> Novozyme 435	6 batches	Methanol stepwise	[40]
Immobilization of lipase onto STY–DVB–PGA	10 batches	Methanol stepwise	[41]
<i>C. antarctica</i> Novozyme 435	50% loss of activity after 24 h	Methanol stepwise	[42]
<i>T. lanuginosa</i> Lipzyme TL-IM and different supports	3 batches of 10 h	Methanol stepwise	[43]
<i>Rhizomucor miehei</i>	16 batches	MMA as substrate	This work
Lipzyme RM IM			

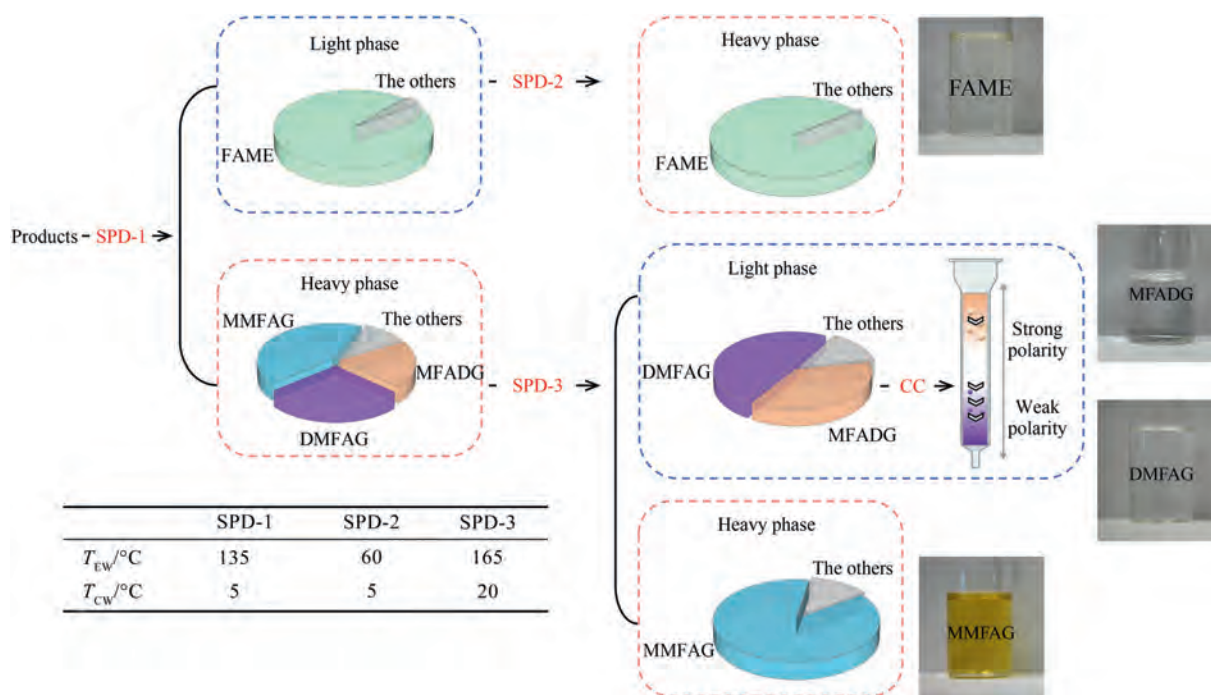


Fig. 7. The scheme of multistep separation process for the reaction mixture. SPD-1 to SPD-3 means three times short-path distillation; CC means silica gel-based column chromatography; the pictures were the photos of each final product. T_{EW} was the temperature of evaporation wall, and T_{CW} was the cooling temperature.

Table 2

Contents of products from each time of short-path distillation and silica gel-based column chromatography.

Trial	SPD-1		SPD-2		SPD-3		CC	
	Light	Heavy	Light	Heavy	Light	Heavy	Strong Polarity	Weak Polarity
Compositions of esters (%)								
TMG	2.80±0.08	--	15.86±0.89	0.66±0.034	--	--	--	--
FAME	96.41±3.81	7.06±0.45	84.14±3.27	98.00±8.63	15.76±0.92	--	0.43±0.11	1.16±0.11
MFADG	0.32±0.01	19.40±1.21	--	0.62±0.01	34.55±2.01	2.60±0.38	97.76±2.84	1.52±0.20
DMFAG	0.37±0.02	26.85±1.30	--	0.57±0.02	46.40±2.10	4.31±0.15	1.21±0.49	95.25±2.89
MMFAG	0.10±0.01	40.90±2.55	--	0.15±0.01	3.29±0.12	81.78±2.57	0.6±0.07	2.07±0.14
TG	--	5.79±0.41	--	--	--	11.31±0.66	--	--

‘--’ indicates not detected or a very low content (≤ 0.1); CC: Column chromatography.**Table 3**

Properties of each product after separation.

Products	Density/ $\text{g} \cdot \text{cm}^{-3}$	Kinematic viscosity at 40 °C/ cSt	T_{onset} /°C	Acid value / $\text{mg} \cdot \text{g}^{-1}$
FAME	0.8728	4.8	243	1.80 ± 0.20
MMFAG	0.9185	35.4	383	0.42 ± 0.05
DMFAG	0.9486	26.3	181	0.54 ± 0.12
MFADG	0.9071	30.2	211	2.41 ± 0.26

Note: 1 cSt = $1 \text{ mm}^2 \cdot \text{s}^{-1}$.

third time SPD (SPD-3, 0.1 Pa, $T_{\text{EW}} = 165 \text{ }^{\circ}\text{C}$, $T_{\text{CW}} = 20 \text{ }^{\circ}\text{C}$) was carried out to make each MFAG separated. However, with the operation of SPD-3, only MMFAG with a higher boiling point could be separated from other components in the high phase. The purity of MMFAG was 81.78%, and the main impurity for the MMFAG was residue TG. The light phase contained of MFADG and DMFAG. Since the two components have the similar boiling point, but difference in the molecular polarity, silica gel-based column chromatography was introduced for further separation. With the further retention time of MFADG on silica gels, MFADG could be effectively separated from DMFAG, the final purity of MFADG and DMFAG reached 97.8% and 95.3%, respectively (Table 2).

The properties assay results of FAME obtained from twice SPD was listed in Table 3. The acid value (1.80 mg KOH/g) was slightly higher was due to the hydrolytic performance of lipase, which could be further improved with alkali refining. Since the contents of glyceride group in their molecule, each kind of MFAGs has a high kinematic viscosity. The onset decomposition temperatures (T_{onset}) of DMFAG, MFADG, and MMFAG are 181, 211, and $243 \text{ }^{\circ}\text{C}$, respectively. This indicates their potential suitability for applications that require high thermal stability. Compared to other two MFAGs products, MMFAG exhibits superior heat resistance, making it more appropriate for high-temperature processing or operational.

4. Conclusions

This study investigates a method for producing biodiesel and fatty acid methacrylate monomers via enzymatic catalysis using MMA and TG. The optimal process parameters were

determined at a system reaction temperature of $50 \text{ }^{\circ}\text{C}$, a molar ratio of MMA to the TG was 4:1, with lipase loading of 7.5% (enzyme: TG, mass) and 0.5% water added, respectively. Meanwhile, the short-path distillation and column chromatographic were employed to effectively separate and purify the complex products, the final purity of FAME, MFADG, DMFAG, and MMFAG were 98.0%, 97.8%, 95.3%, and 81.8%, respectively. Compared to the traditional biodiesel production with methanol application, MMA demonstrates lower toxicity to lipases, allowing for straightforward, once addition of all the substrates without complex addition flow control, relatively fast equilibrium speed, simplifying the process, and enhancing operational feasibility. The co-production strategy not only yielded high-quality biodiesel but also generated methacrylic acid derivatives that may be used in various industrial applications such as paints, varnishes, and coatings. This method hence offers a promising new direction for the biodiesel industry, establishing a more sustainable and economically efficient production approach. This research lays the foundation for the transformation of the biodiesel production industry, aligning with global sustainable development goals.

CRediT Authorship Contribution Statement

Dong Lu: Writing – original draft, Software, Methodology, Investigation, Data curation. Shuming Jin: Software, Investigation, Data curation. Qiuyang Wu: Writing – review & editing, Formal analysis. Jiahao Liu: Formal analysis. Fang Wang: Supervision. Li Deng: Writing – review & editing, Supervision, Funding acquisition. Kaili Nie: Writing – review & editing, Supervision, Funding acquisition.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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