Effect of the Formation of Diglycerides/Monoglycerides on the Kinetic Curve in Oil Transesterification with Methanol Catalyzed by Calcium Oxide

Xiaobing Chen, Zhenhua Li, Yuan Chun,* Fan Yang, Haocheng Xu, and Xingcai Wu*

ABSTRACT: Many researchers reported that a sigmoid kinetic curve was obtained in oil transesterification with methanol catalyzed by CaO and gave different explanations for this formation. In this paper, heterogeneously catalyzed transesterification of soybean oil with methanol using CaO has been investigated. The solid catalyst and the liquid reaction mixture under different reaction time periods were characterized by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, and high-performance liquid chromatography (HPLC) to reveal the formation of an S-shape kinetic curve. The appearance of calcium hydroxide, calcium methoxide, calcium glyceroxide, fatty acid calcium, diglycerides, and monoglycerides and their contributions to the kinetic curve have been discussed. The low reaction rate in the induction period can be attributed to mass transfer in this three-phase system. However, the formation of surfactants, diglycerides and monoglycerides, promotes the emulsification of the reaction mixture and numerous emulsion reactors are generated. These emulsion reactors can improve the contact of the solid catalyst with the reactants and thus accelerate the reaction.

1. INTRODUCTION

Due to the rapid development of the modern industry and service industry, the serious problems of environment pollution and energy crisis force researchers to look for new energy sources. As a new replacement, biodiesel has the available excellent advantages of renewability, high octane number, high flash point, good lubricity, low viscosity, and high biodegradability; meanwhile, it is able to reduce the emissions of environmental pollutants.1−3 Biodiesel is closer to traditional diesel than other energy sources, which can be used directly for internal combustion engines without any transformation. Therefore, many countries around the world are working on the synthesis of biodiesel.

From the economic point of view, much interest has been focused on catalysing the vegetable oil transesterification.1−7 These synthesis methods include acid/base catalysis, enzyme catalysis, and supercritical fluid method, and homogeneous acid/base catalysis has been widely employed in industrial processes due to the high activity and affordable price.8−14 However, the acid/base catalysis using homogeneous catalysts still have several drawbacks: (a) the catalyst cannot be recycled; (b) equipment should be resistant to strong acids and bases; and (c) waste water produced during purification and separation will bring about environmental pollution.15 In contrast, the substitution of homogeneous catalysts by heterogeneous ones may allow achieving more efficient and economical biodiesel production processes.16,17 Compared to the solid acid catalyst, solid base catalyst has received extensive attention due to the fact that it can form a high concentration of methyl ester in lower temperatures and shorter time periods. Many kinds of solid base materials, such as an intrinsic solid base, supported solid base, composite, alkali metal ion exchange zeolite, and anion exchange resin, have been employed to catalyze this reaction.1,18−25 Among them, CaO is one of the most promising catalysts due to its low solubility in methanol, low cost, and availability.1,26−28

A sigmoid kinetic curve is usually observed in heterogeneously catalyzed transesterification of vegetable oils with methanol using a CaO catalyst, which is a three-phase reaction system due to the poor solubility of methanol in oil. The kinetic curve can be divided into three segments: an initial mass-transfer-controlled segment (slow), a chemically controlled segment (fast), and a final segment close to equilibrium (slow).5,29,30 It is known that the interfacial mass-transfer resistance has an apparent effect on the conversion of oil in this three-phase reaction system; methanol is in fact not effectively
used for the reaction. There are different opinions proposed for the transition from the initial mass-transfer-controlled segment to chemically controlled segment. Many researchers report that other more active Ca species are generated during transesterification. Arzamendi et al.31 proposed that the real active sites are Ca(OH)2 that are formed from CaO and H2O. Venkat Reddy et al.32 believed that calcium methoxide (CaM) is the catalytic active substance since alcohol is readily reactive with calcium oxide. Kouzu et al.33 and Esipovich et al.28 reported that calcium glyceroxide (CaD) was formed during the transesterification of vegetable oil, which is more active than the CaO catalyst. The formation of these more active Ca species can accelerate the transesterification reaction, which potentially contributes to the S-shape kinetic curve.16,34 Likozar et al.30 suggested that the rotational speed has the most profound influence on the duration of the transport phenomena-limited region. Some researchers have pointed out that a soluble substance leached away from calcium oxide during transesterification, which are rather active and have contribution to the transesterification.35,36 For homogeneously catalyzing oil methanolsysis, the S-shape kinetic curve is ascribed to the change of reaction mixture, i.e., the transition from two-phase to a single-phase system during the reaction.37

In this paper, the reason for the formation of the S-shape kinetic curve, especially for the transition from the initial mass-transfer-controlled segment to chemically controlled segment, in vegetable oil transesterification using CaO was investigated. For this purpose, the CaO catalyst and liquid reaction mixture under different treatments were characterized by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, and high-performance liquid chromatography (HPLC) to measure the conversion of CaO solid and the change of liquid mixture component. The reaction result was compared with those on several possibly formed active Ca species. Also, the mixing state of oil, methanol, and CaO with or without adding diacylglycerides (DG) and monoacylglycerides (MG) was studied. Based on the experiments results, the S-shape kinetic curve should be mainly ascribed to the formation of DG and MG during the reaction, which function as surfactants to promote the formation of numerous emulsion reactors and thus decreases the mass transfer effect.

2. RESULTS AND DISCUSSION

2.1. Catalytic Behavior of Calcium Oxide. The transesterification of soybean oil with methanol was carried out at 343 K using CaO as a catalyst, which has a specific surface area of 5 m2/g (data not given in tables or figures), and the reaction result is displayed in Figure 1. CaO shows moderate activity for this transesterification at 343 K, and the fatty acid methyl esters (FAMEs) yield exceeds 80% in a 3 h run. Detail observation can find that the kinetic curve is divided into three segments, with reaction periods from 0 to 0.5 h, 0.5 to 2 h, and 2 to 3 h. The reaction rate has an apparent acceleration after a 0.5 h run, in agreement with previous reports.16,38 The kinetic curve at 323 K also shows an apparent acceleration after the 1 h run, and the conversion of soybean oil is lower than that at 343 K, due to a lower reaction rate. The spent CaO catalyst was separated out after reaction at 343 K for 3 h and washed with methanol. After drying, the spent catalyst was also used to catalyze this reaction, and the reaction result is also shown in Figure 1. The initial conversion rate of soybean oil on this spent catalyst is apparently higher than that of the fresh one. It indicated that the active sites in the catalyst have been changed after transesterification for 3 h.19,40

Figure 2 depicts the effect of water content on the catalytic activity of CaO. The initial FAME yield shows a little change when water is present in the reaction mixture, but the yield has an apparent increase in the 1 h run, which is due to the formation of surface OH− species. The FAME yield curves on these water-added reactions are very similar to those on Ca(OH)2, indicating that CaO is possibly converted into Ca(OH)2 to catalyze this reaction if water is present in the reaction mixture. However, the FAME yield decreases dramatically if 30 mol water per mole CaO is added, which can be ascribed to the hydrolysis of FAME in the presence of too much water followed by saponification.41

Figure 3 shows the catalytic activities of CaO pretreated by methanol, glycerol, and their mixture. The FAME yield apparently increases when CaO is pretreated with methanol at 343 K for 3 h (Figure 3A), and the FAME yield curve is similar to that for CaM. In fact, this pretreatment procedure is almost the same as that of CaM synthesis. However, pretreatment of CaO with glycerol or the mixture of glycerol

![Figure 1](https://dx.doi.org/10.1021/acsomega.9b04431)
ACS Omega 2020, 5, 4646−4656
and methanol decreases the catalytic activity of CaO (Figure 3B). Detailed observation can find that the FAME yield decreases with the increase of glycerol content and the induction period extends accordingly. It seems to suggest that the formation of byproduct glycerol during the ester transformation is disadvantageous for the conversion of oil. On the other hand, the catalytic activity increases when glycerol-treated CaO is washed with methanol before adding it to the reaction mixture, and the FAME yield curve is very similar to that for CaD. It means that the pretreatment of CaO with glycerol can generate CaD at least on the surface of CaO, but the excess glycerol will cover the surface of CaD and decrease the activity.

2.2. Characterizations of Catalysts. Figure 4 depicts the XRD patterns of fresh and spent CaO. The XRD pattern of fresh CaO shows several peaks at 32.4, 37.5, 54.0, 64.3, and 67.5°, which can be readily assigned to a pure phase of CaO (JCPDS card number 37-1497). The characteristic peaks of CaO disappear on spent CaO, and some new diffraction peaks at 8.5, 10.4, and 21.4° are clearly visible, which can be assigned to the crystalline phase of CaD.42 It indicates that most of CaO is transformed into CaD with the byproduced glycerol after the reaction.

To better understand the transformation of CaO during the transesterification of soybean oil, the solid catalyst was separated and analyzed by XRD after reaction for various times, and the result is shown in Figure 5. The characteristic peaks of Ca(OH)2 at 18.0, 20.8, and 34.1° are visible in the 0.5 h run, and their intensities almost remain unchanged during the reaction. The characteristic peaks of CaD at 8.4 and 10.3° appear in the 1.5 h run, and their intensities increase rapidly with an increase in reaction time. Accordingly, the intensities of characteristic peaks of CaO decrease and the solid catalyst consists mostly of CaD after reaction in the 3 h run. It indicates that the CaO catalyst has been changed during transesterification. Part of CaO particles are transformed to Ca(OH)2 particles in the initial stage, and most of them are transformed to CaD in the last stage.

The FT-IR spectra of various Ca species are presented in Figure 6A. It can be found that FT-IR spectra of CaO, Ca(OH)2, CaM, and CaD have significant difference in the wavenumber range between 800 and 1600 cm⁻¹. The FT-IR spectrum of CaO shows nearly baseline in this wavenumber range. The FT-IR spectrum of Ca(OH)2 shows the characteristic bands at 1423 and 874 cm⁻¹, which can be ascribed to the O−H deformation vibration of −OH groups bonded to calcium and the C−O bond, respectively.16 An intense band at 3645 cm⁻¹ corresponding to the O−H stretching vibration of −OH groups can also be distinguished from the FT-IR spectrum of Ca(OH)2. The FT-IR spectrum of CaM shows a
strong absorption band at 1054 cm\(^{-1}\) and a weak broad band at 1465 cm\(^{-1}\), which are assigned to the stretching vibration of the C–O bonds and bending modes of C–H bonds, respectively.\(^{28}\) More bands can be distinguished from the FT-IR spectrum of CaD; they are the bands assigned to the various bending modes of C–H bonds (1470, 1446, 1265, 1230, 946, and 915 cm\(^{-1}\)), the bending modes of C–O–H bonds (1378 and 1308 cm\(^{-1}\)), the stretching mode of C–O bonds (1129 and 1075 cm\(^{-1}\)), and the stretching mode of C–C bonds (860 cm\(^{-1}\)).\(^{39}\) Thus, it is easy to distinguish the change of surface species of the catalyst during the reaction by FT-IR spectra.

Figure 6B depicts the change of FT-IR spectra of the CaO catalyst during the transesterification of soybean oil. The bands at 3645, 1436, and 865 cm\(^{-1}\) appear after reaction for 10 min, demonstrating that Ca(OH)\(_2\) is formed at the beginning of the reaction. The intensities of these bands are enhanced after reaction for 0.5 and 1 h; however, they decrease in 1.5 h run, which possibly results from the further transformation or the effect of the formation of other species. The bands for CaD appear after reaction for 2 h, and their intensities have no apparent change with reaction time prolonging to 3 h, in agreement with the above XRD results.

It is surprising to find that the band at 1054 cm\(^{-1}\) is pronounced when CaO is treated with methanol at 343 K (CaM), while it is hardly distinguished from the FT-IR spectrum of the CaO catalyst during the transesterification of soybean oil with methanol. It indicates that the presence of soybean oil hinders the formation of CaM.

2.3. Discussion on the Formation of the S-Shape Kinetic Curve. During the transesterification of soybean oil, it can be found that the reaction rate has an apparent change after the 0.5 h run, showing the S-shape kinetic curve, in agreement with the previous reports.\(^{16,39}\) Due to the presence of three phases in the reaction mixture and the porous character of the solids, most of the researchers ascribe the sigmoid kinetic curves to the existence of mass transfer limitations for the initial reaction period followed by a kinetic control for the later period.\(^{16,30}\) To confirm the effect of mass transfer, various stirring speeds were used for this reaction, and the reaction result is shown in Figure 7. It can be found that a higher stirring speed results in a faster reaction rate and hardly any FAME is formed without stirring. It indicates that mass transfer affects the conversion of oil seriously.\(^{30}\)

Many researchers ascribe the acceleration of reaction after the induction period to the formation of new active species, such as Ca(OH)\(_2\), CaM, and CaD.\(^{16,28,39}\) Some even ascribe this to the effect of leaching.\(^{35}\) To estimate the effect of leaching, the reaction mixtures in 0.5 and 0.9 h runs were centrifuged, respectively, and the liquid phases were reused for reaction without adding the catalyst. As shown in Figure 8, the FAME yields almost keep unchanged on these reaction systems (curves d and e), indicating that the effect of the leaching effect can be ignored. Lópe Granados et al.\(^{43}\) also confirmed that the total homogeneous contribution is much smaller than that arising from the heterogeneous sites provided that the catalyst loading is large enough.

Glycerol is the byproduct of the transesterification of soybean oil. The pK\(_a\) of glycerol is 14.15, which is lower than 15.5 of methanol; thus, it can react with CaO, a solid base. Based on the analysis of the IR spectra, Esipovich et al.\(^{28}\) have suggested that CaO first reacted with methanol and caused the formation of CH\(_3\)O\(^{-}\) and OH\(^{-}\) groups on the
with methanol at 343 K. The reaction was first carried out at 343 K for 0.5 h; the solid and liquid reaction mixtures were separated by centrifugation; and then (a) solid was added to new reactants, (b) liquid reaction mixture was added to a new CaO catalyst, (c) solid and liquid reaction mixtures were stirred again for further reaction, (d) liquid reaction mixture was stirred for further reaction, and (e) liquid reaction mixture separated from the reaction system in the 0.9 h run was stirred for further reaction.

oxide surface, and these groups were further replaced by glycerol with the formation of a more stable CaD. CaD exhibits a catalytic performance higher than that of the commercial CaO. It is worth noting that a higher content of glycerol is needed to generate CaD in this reaction mixture. As shown in XRD patterns (Figure 5) and FT-IR spectra (Figure 6), most of the CaO is transformed into CaD in the 2 h run; however, hardly, any CaD can be distinguished in the 1 h run due to the lower content of glycerol, in agreement with Kouzu’s report. It indicated that the acceleration of reaction after the 0.5 h run results not from the formation of CaD. CaM also shows higher initial activity than that of CaO in this transesterification, and it can be formed by a reaction between CaO and methanol at 343 K. However, the existence of soybean oil seriously hinders the formation of CaM and hardly any signal of CaM is observed in the XRD patterns or IR spectra of the catalyst in the 0.5 or 1 h run. Different from CaD and CaM, the results from IR spectra and XRD patterns show that Ca(OH)₂ is formed at the beginning of the reaction and preserved during the reaction. The formation Ca(OH)₂ possibly results from the reaction of CaO with the trace water in reactants. However, although the yield of FAME on Ca(OH)₂ is higher than that on CaO in the 1 h run, the yield of FAME on both catalysts is similar in the 0.5 h run and the induction period is still present in the kinetic curve (Figure 2). Moreover, as shown in Figure 8, the catalyst after the 0.5 h run was separated and reused for the reaction; the catalytic activity and tendency are similar to those on fresh CaO (curve a). It indicates that the acceleration of reaction after the induction period results not from the conversion of CaO to other active species.

It is worth noting that the induction period disappears when the reaction mixture in the 0.5 h run was separated by centrifugation and remixed for reaction, and the FAME yield in next 0.5 h run is similar to the original result in 1 h run (curve c in Figure 8). Moreover, if the liquid reaction mixture was used for reaction with the new CaO catalyst, the induction period also disappears (curve b in Figure 8). It indicates that the composition of the liquid reaction mixture has been changed after the 0.5 h run. Csernica et al. has investigated the homogeneous catalyzed oil methanolysis by KOH and found that soybean oil is actually soluble in methanol and the rate of mass transfer and the equilibrium concentration of oil in methanol increases with an increase in methyl ester concentration. Thus, they ascribe the S-shape kinetic curve to the transition from a two-phase to a single-phase system in this homogeneous reaction due to the formation of methyl esters. However, the yield of methyl esters is about 3.4% in the 0.5 h run using CaO as the catalyst, which is too low to improve the solubility of oil in methanol apparently. As shown in Figure 9, the initial activity has hardly any change when

3.4% of methyl esters is directly added into the reaction system before the reaction, which shows the evidence that the acceleration of reaction after induction period derives not from the formation of methyl esters during the reaction.

Fatty acid calcium is one of the possible compositions in the liquid reaction mixture, which can be produced via neutralization of the catalyst by free fatty acid (FFA) or saponification during the transesterification of soybean oil. Unlike waste frying oils, the content of FFA is usually around 0.2% in soybean oil and the effect of the neutralization can be ignored. It has been reported that the saponification of the FAME molecules and glycerides happens simultaneously during the transesterification of triglycerides (TG) catalyzed by CaO itself. The FAME formation rate is faster than that for Ca soap formation, and the total content of Ca₄ in the ester and alcoholic phases is only 0.13 wt %, compared to more than 90% of the FAME yield. The yield of FAME is only 3.4% in the 0.5 h run, and thus, the formation of Ca soap is very limited at this time. Puna et al. have reported that the S-shape kinetic curve is still present using waste frying oils as a reactant, which has a content of FFA of 3.7%. It indicates that the formation of a small amount of fatty acid calcium would not accelerate the transesterification reaction. In fact, the formation of fatty acid calcium should be responsible for the deactivation of the CaO catalyst.
It is worth noting that MG and DG are also formed in this transesterification reaction. The boiling points of these molecules are too high to be detected by GC, and thus, the reaction mixtures are also analyzed by HPLC.

Figure 10 depicts the HPLC chromatographs of the reaction mixtures at different time periods. Generally, four groups of peaks can be distinguished in the HPLC chromatographs of the reaction mixtures; they are peaks in the region between 0 and 4 min attributed to MG and free fatty acids, between 4 and 6 min attributed to methyl esters, between 6 and 12 min attributed to DG, and longer than 20 min attributed to TG. It can be found that methyl esters (FAME), MG and DG, are formed at the beginning of the reaction. The content of methyl esters increases along the reaction, in agreement with the GC results. While the content of DG and MG reaches maximum in 1 h run, it decreases with the further reaction. The ratios of these products at different reaction time periods are shown in Figure 11. It can be found that both the ratios of DG/FAME and (DG + MG)/FAME reach maximum after 0.5 h run, and they are much higher than 1. It is thus very possible that the formation of DG and MG, especially DG, has more significant effect on the reaction rate than methyl esters at the beginning of the reaction. As shown in Figure 9, the induction period disappears when the DG and MG, which share the same amount as that generated in the 0.5 h run from HPLC quantitative analysis, are added into the reaction system, and the FAME yield in the 0.5 h run is very similar to the original result in the 1 h run. Therefore, the formation of MG and DG should be responsible for the acceleration of reaction rate, i.e., the formation of the S-shape kinetic curve.

López Granados et al. have reported that forming a slurry by mixing CaO with biodiesel results in both protection against poisoning by atmospheric CO₂ and H₂O and an increase in reaction rate. They ascribe it to the presence of minute amounts of MG and/or DG in biodiesel, and the transesterification of MG and DG during the pretreatment with methanol results in the release of glycerol, which then reacts with the catalyst surface, resulting in the formation of very active surface CaD species. Thus, a few minutes are needed to treat the slurry with methanol before proceeding with the transesterification reaction. In the present study, as discussed above, hardly any CaD can be detected on the solid catalyst in the 0.5 h run and the solid catalyst after the 0.5 h run shows an activity similar to that of fresh CaO. Moreover, when 0.37 g of glycerol (~1 wt % of oil added), which is higher than the theoretical value of glycerol produced in the 0.5 h run, was added into the reaction mixture, the FAME yield in 0.5 h run was still lower than 5% (Figure 9). It fully indicates that the formation of CaD by a reaction of DG or/and MG with methanol is not the main reason for the acceleration of the transesterification reaction after the 0.5 h run.

Both the hydrophobic long-chain alkyl and hydrophilic carboxyl groups are present on DG and MG molecules, and they are typical surfactants. It is well known that adding a surfactant is advantageous in mixing water and oil, and thus, it is very possible that the formation of DG and MG improves the mixing of soybean oil and methanol. As shown in Figure 12, certain amounts of soybean oil and methanol were evenly added to two glass tubes; the left tube also contains small amounts of DG and MG. Both of tubes were shaken for 1 min, followed by standing for a long time. It can be found that the methanol and soybean oil are separated completely in 10 min without adding DG and MG. However, the methanol and soybean oil are hardly separated after adding DG and MG; the oil phase (lower phase) is still turbid even after 24 h standing. It indicates that the presence of DG and MG promotes the formation and maintaining of the emulsion. On the other hand, the volume of the upper phase (methanol) decreases after separation, indicating more methanol molecules are dissolved into soybean oil in the presence of DG and MG. Figure 12 also shows the dispersion state of CaO in oil and methanol with or without adding DG and MG. It can be found that CaO is dispersed in oil rather than methanol by stirring. Without adding DG/MG, methanol is quickly separated from the oil phase; however, the separation rate decreases dramatically when DG/MG is added; almost half of methanol locates in the oil phase in emulsion morphology after standing for 10 min.

Moreover, photomicrographs of the stable oil phase further confirmed the existence of the emulsion. As shown in Figure 13, occasionally, one bubble with a diameter of around 40 μm can be seen in the visual field of pure oil after shaking for 1 min followed by standing for 0.5 h, and the bubble almost disappears after 1 h standing. A spherical bubble or droplet is also seldom observed in the oil phase of the oil–methanol

![Figure 10](https://pubs.acs.org/doi/abs/10.1021/acsomega.9b04431)

**Figure 10.** HPLC chromatographs of the reaction mixtures at different reaction time periods for the transesterification of soybean oil with methanol on CaO (catalyst/oil = 1.5 wt %, methanol/oil = 9:1).

![Figure 11](https://pubs.acs.org/doi/abs/10.1021/acsomega.9b04431)

**Figure 11.** Ratios of different products and yields of DG and MG as a function of reaction time on CaO for the transesterification of soybean oil with methanol at 343 K (catalyst/oil = 1.5 wt %, methanol/oil = 9:1).
Figure 12. Photographs of the mixture of soybean oil and methanol with (left) and without (right) adding DG/MG (a–c). Both mixtures were shaken vigorously for 1 min, followed by standing for 10 min (a), 9 h (b), and 24 h (c). Photographs of the mixture of soybean oil, methanol, and CaO with (left) and without (right) adding DG/MG (d–f). Both mixtures were shaken vigorously for 1 min, followed by standing for 2 min (d), 5 min (e), and 10 min (f).

Figure 13. Photomicrographs of the oil phase of several systems after shaking vigorously for 1 min, followed by standing for 0.5 h (left) and 1 h (right). (a) Soybean oil; (b) soybean oil and methanol; (c) soybean oil, methanol, and DG/MG; (d) soybean oil, methanol, and CaO; and (e) soybean oil, methanol, CaO, and DG/MG.
mixture upon similar treatment, indicating that droplets of methanol in oil phase are unstable. However, many spherical liquid droplets with diameters ranging from 1.9 to 11.7 μm exist in the oil phase of the oil–methanol mixture containing DG/MG, even after standing for 1 h. Since DG and MG are completely dissolved in oil in our condition, the droplets should derive from methanol, which is consistent with the decrease of the methanol volume upon this treatment (Figure 12). If CaO was added to the mixture of oil and methanol, many irregular solid particles ranging from 3.9 to 19 μm would be observed in the oil phase even after standing for 1 h, as shown in Figure 13d. It indicates that the dispersion of small CaO particles in oil is stable. However, few spherical liquid droplets can be observed after 0.5 h standing, indicating that the presence of CaO did not improve the dispersion of methanol in oil. In the case of the mixture consisting of oil, methanol, DG/MG, and CaO, plenty of spherical droplets and irregular solid particles can be observed after 0.5 or 1 h standing, indicating that a large amount of CaO catalysts and methanol is present in soybean oil. More importantly, many of the methanol droplets and CaO particles contact with each other and thus a number of emulsion reactors are generated. Therefore, the mixing state of oil, methanol, and CaO is greatly improved in the presence of DG/MG.

At the beginning of the reaction, the reaction mixture contains a few DG/MG, the CaO catalyst locates in the soybean oil phase, and the dispersion state of methanol in the oil phase is poor, which seriously hinders the contact of methanol with CaO. Thus, the transesterification rate is low in the initial reaction period on the CaO catalyst. However, DG and MG are formed in the 0.5 h run, which greatly improve the mixing state of reactants and the solid catalyst. Numerous emulsion reactors are generated in the reaction system, in which both the liquid reactants can well contact with the CaO solid, and thus, the effect of mass transfer is decreased. Therefore, the reaction is accelerated in the 0.5 h run.

It should be noted that the induction period can hardly distinguished from the FAME yield curve on the spent catalyst. As discussed above, this spent catalyst comprises mainly of CaD. Although the specific surface area of CaD (4 m²/g) is similar to that of CaO (5 m²/g) (data not given in tables or figures), a particular interaction between glyceroxide and calcium ions is present on the CaD catalyst, which creates a surface with hydrophobic and hydrophilic sites and a hydrogen bond network favouring the approach of methanol and triglyceride molecules.39 Lukić et al.52 have reported that the presence of CaD as a catalyst in methanolysis of soybean oil is poor, which seriously hinders the contact of methanol with CaO with methanol. Thus, the transesterification rate is low in the initial reaction period on the CaO catalyst. However, DG and MG are formed in the 0.5 h run, which greatly improve the mixing state of reactants and the solid catalyst. Numerous emulsion reactors are generated in the reaction system, in which both the liquid reactants can well contact with the CaO solid, and thus, the effect of mass transfer is decreased. Therefore, the reaction is accelerated in the 0.5 h run.

3. CONCLUSIONS

The heterogeneously catalyzed transesterification of soybean oil with methanol using CaO presents an S-shape kinetic curve. The low reaction rate in the induction period derives mainly from the mass transfer since a three-phase system is used for this transesterification. DG and MG are formed at the beginning of the reaction, which are mainly responsible for the acceleration of the reaction rate after the 0.5 h run. These intermediates (MG and DG) function as surfactants to promote the formation of numerous emulsion reactors, which are helpful to the contact of the solid catalyst with liquid reactants, and thus decrease the effect of mass transfer.

4. EXPERIMENTAL SECTION

4.1. Materials. CaO was purchased from Shanghai Chemical Reagent, China, which was calcined at 973 K for 3 h in N₂ flow and then cooled to room temperature in a N₂ atmosphere prior to use. Methanol (HPLC) and glycerol (A.R.) were supplied by Nanjing Chemical Reagent, China. DG and MG were supplied by Shanghai Aladdin Bio-Chem Technology Co., Ltd., China. C₁₂H₂₅COOCH₃ was supplied by Tokyo Chemical Industry Co. Ltd. (TCI), Japan. Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly-(ethylene glycol) (P123, Mₙ = 1100) was obtained from Aldrich. The biodiesel for the treatment was obtained by methanolysis of soybean oil. CaM was prepared by reacting CaO with methanol. The methanol and calcined CaO at a molar ratio of 40 were treated at 343 K for 3 h with a reflux condenser. Then, the filtered solid was dried at 373 K in N₂ flow. CaD was obtained by treating calcined CaO with 4 times more glycerol, using methanol as solvent. The typical procedure was similar to the synthesis of CaM, and the excess glycerol was removed by washing with methanol before drying. Ca(OH)₂ was prepared by the reaction of calcined CaO with excess water at room temperature for 12 h and dried at 373 K in N₂ flow.

4.2. Catalyst Characterizations. X-ray diffraction (XRD) patterns of samples were recorded on a Rigaku D/max-RA diffractometer with Cu Kα radiation in the 2θ range from 10 to 80°. FT-IR spectra were recorded on a Bruker ALPHA infrared spectrometer operated in attenuated total reflection (ATR) mode. The number of scans was set at 64 with a resolution of 2 cm⁻¹, over the range 4000–600 cm⁻¹. The solid catalyst after reaction at different time periods was filtered and washed with methanol and acetic ether to remove any liquid compounds that were physically absorbed on the solid and dried at 373 K in N₂ flow. Adsorption of N₂ was measured at 77 K using a Micromeritics ASAP 2020 system. Prior to testing, the sample was evacuated at 573 K for 4 h.53 The specific surface area was calculated based on the Brunauer–Emmett–Teller (BET) equation with adsorption data in the relative pressure range from 0.05 to 0.3. The photomicrographs of the dispersion of methanol and/or CaO in oil phase were taken by a JS94H micro-electrophoresis meter. Certain amounts of methanol, oil, DG/MG, and CaO, with the same ratio for the transesterification reaction, were shaken vigorously for 1 min and allowed to stand for 0.5 and 1.0 h before observation. The magnification factor of the lens is 4.32 pixel/μm.

4.3. Transesterification of Soybean Oil with Methanol. The transesterification of soybean oil with methanol was performed in a 250 mL two-necked flask with a reflux condenser and a magnetic stirrer under atmospheric pressure,
and the repeated measurements showed the error of yield within 3%. Typically, 12 g of methanol and 35.5 g of soybean oil (methanol/oil molar ratio is 9:1) were placed in the flask. Then, 0.56 g of catalyst, which had been calcined at 973 K for 3 h in N₂ flow, was added into the mixture and heated by a water bath at 343 K at a stirring speed of 1200 rpm. At the intervals of 0.5 h, a few amounts of reaction mixture were withdrawn and extracted with 0.1 M HCl and water to remove methanol and catalyst. After centrifugation, the upper liquid (FAME and unreacted soybean oil) and methyl heptadecanoate/n-hexane solution were mixed. With the internal standard method using gas chromatography (GC), the FAME yield was calculated by

\[
y_{\text{FAME}} = \frac{\sum_{i=m}^{5} y_i A_i}{\sum_{i=m}^{5} A_i} \times \frac{m_{\text{MH}}}{m} \times 100\%
\]

Here, \(\sum_{i=m}^{5} y_i A_i\) is the corrected peak area of the FAMEs, \(A_{\text{MH}}\) is the peak area of methyl heptadecanoate, \(y_i\) is the correction factor of each methyl ester, and \(m\) and \(m_{\text{MH}}\) are the masses of upper liquid methyl and methyl heptadecanoate, respectively.

Pretreatments of CaO with methanol and glycerol in methanol were carried out as follows: 0.56 g of calcined CaO was placed in a 50 mL two-necked flask and 12 g of methanol containing various amounts of glycerol was added into the flask. After 3 h of stirring at 343 K, 35.5 g of soybean oil was added to perform the transesterification. Pretreatment of CaO with glycerol was carried out as follows: 0.56 g calcined CaO was placed in a 50 mL two-necked flask, and 3.82 g glycerol was added into the flask. After 3 h of stirring at 343 K, 12 g methanol and 35.5 g soybean oil were added to perform the transesterification. For comparison, the pretreated CaO catalysts were also washed with methanol, filtered and dried at 373 K in flowing of N₂. The resulting solid was then added to the mixture of 12 g methanol and 35.5 g soybean oil for the transesterification.

HPLC was used to detect the presence of the fatty acid (FA), MG, DG, triglycerides (TG), and the methyl esters using a Agilent 1260 Infinity II equipped with a UV–vis detector, 6120 quadrupole liquid chromatography/mass spectrometry (LC/MS) system, and Poroshell 120 EC-C18 column (2.7 μm) (3 × 150 mm²). The samples were diluted with a 10% solution of 5:4 (v/v) isopropyl alcohol/hexane. HPLC-grade methanol was used in line A, and a solution of 5:4 (v/v) isopropyl alcohol/hexane was used in line B. The elution was performed isocratically with 90% A and 10% B at 50 °C, with a flow rate of 1 mL/min, and the wavelength was set to 205 nm.16

### AUTHOR INFORMATION

**Corresponding Authors**

**Yuan Chun** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China; orcid.org/0000-0003-4425-3862; Email: ychun@nju.edu.cn

**Xingcai Wu** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China; orcid.org/0000-0001-9815-0557; Email: wuxingca@nju.edu.cn

**Authors**

**Xiaobing Chen** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

**Zhenhua Li** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

**Fan Yang** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

**Haocheng Xu** – Key Laboratory of Mesoscopic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b04431

**Author Contributions**

X.C. and Z.L. contributed equally to the realization of the manuscript. The manuscript was written through contributions of all authors. All authors have given final approval to the version of the manuscript.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the National Science Foundations of China (Nos. 21573104 and 21673108), and the testing fund of Nanjing University.

**REFERENCES**

1. Lee, A. F.; Wilson, K. Recent developments in heterogeneous catalysis for the sustainable production of biodiesel. Catal. Today 2015, 242, 3–18.

2. Zabeti, M. D.; Wan, W. M. A.; Aroua, M. K. Activity of solid catalysts for biodiesel production: A review. Fuel Process. Technol. 2009, 90, 770–777.

3. Sivasamy, A.; Cheah, K. Y.; Fornasiero, P.; Kemausuor, F.; & et al. Catalytic applications in the production of biodiesel from vegetable oils. ChemSusChem 2009, 2, 278–300.

4. Lee, A. F.; Bennett, J. A.; Manayil, J. C.; & Wilson, K. Heterogeneous catalysis for sustainable biodiesel production via esterification and transesterification. Chem. Soc. Rev. 2014, 43, 7887–7916.

5. Sharma, Y. C.; Singh, B.; Upadhyay, S. N. Advancements in development and characterization of biodiesel: A review. Fuel 2008, 87, 2355–2373.

6. Chen, G.-Y.; Shan, R.; Yan, B.-B.; Shi, J.-F.; Li, S.-Y.; & Liu, C.-Y. Remarkably enhancing the biodiesel yield from palm oil upon abalone shell-derived CaO catalysts treated by ethanol. Fuel Process. Technol. 2016, 143, 110–117.

7. Ye, W.; Gao, Y.; Ding, H.; Liu, M.; Liu, S.; Han, X.; & Qi, J. Kinetics of transesterification of palm oil under conventional heating and microwave irradiation, using CaO as heterogeneous catalyst. Fuel 2016, 180, 574–579.

8. Ma, F.; Hanna, M. A. Biodiesel production: a review. Bioresour. Technol. 1999, 70, 1–15.

9. Kawashima, A.; Matsubara, K.; Honda, K. Acceleration of catalytic activity of calcium oxide for biodiesel production. Bioresour. Technol. 2009, 100, 696–700.

10. Lotero, E.; Liu, Y.; Lopez, D. E.; Suwannakarn, K.; Bruce, D. A.; Goodwin, J. G., Jr. Synthesis of biodiesel via acid catalysis. Ind. Eng. Chem. Res. 2005, 44, 5353–5363.

11. Enweremadu, C. C.; Mbarawa, M. M. Technical aspects of production and analysis of biodiesel from used cooking oil-A review. Chem. Res. Ind. Eng. 2015, 2355–2373.
(12) Dhanaut, J.; Daquin, J. P.; Lee, A. F.; Wilson, K. Hierarchical macroporous–mesoporous SBA-15 sulfonic acid catalysts for biodiesel synthesis. Green Chem. 2010, 12, 296–303.
(13) Ren, Y.; He, B.; Yan, F.; Wang, H.; et al. A continuous process for biodiesel production in a fixed bed reactor packed with cation-exchange resin as heterogeneous catalyst. Bioresour. Technol. 2012, 113, 19–22.
(14) Lopiano, P.; Bernal, J. M.; Vautlier, M. Towards continuous sustainable processes for enzymatic synthesis of biodiesel in hydrophobic ionic liquids/supercritical carbon dioxide biphasic systems. Fuel 2011, 90, 3461–3467.
(15) Atadashi, I. M.; Aroua, M. K.; Aziz, A. A. Biodiesel separation and purification: a review. Renewable Energy 2011, 36, 437–443.
(16) Soares Dias, A. P.; Puna, J.; Gomes, J.; Correia, M. J. N.; Bordado, J. Biodiesel production over lime. Catalytic contributions of bulk phases and surface Ca species formed during reaction. Renewable Energy 2016, 99, 622–630.
(17) Sani, Y. M.; Daud, W. M. A. W.; Abdul Aziz, A. R. Activity of solid acid catalysts for biodiesel production: A critical review. Appl. Catal., A 2014, 470, 140–161.
(18) Lukić, I.; Krstić, J.; Jovanović, D.; Skala, D. Alumina/silica supported K2CO3 as a catalyst for biodiesel synthesis from sunflower oil. Bioresour. Technol. 2009, 100, 4690–4696.
(19) Xie, W.; Huang, X.; Li, H. Soybean oil methyl esters preparation using NaX zeolites loaded with KOH as a heterogeneous catalyst. Bioresour. Technol. 2007, 98, 936–939.
(20) Volli, V.; Purkait, M. K. Selective preparation of zeolite X and A from fly ash and its use as catalyst for biodiesel production. J. Hazard. Mater. 2015, 297, 101–111.
(21) Creasey, J. J.; Chiaregato, A.; Manayil, J. C.; Parlett, C. M. A.; Wilson, K.; Lee, A. F. Alkaline and alkaline-earth metals compounds as catalysts for biodiesel synthesis from rapeseed oil. Energy Environ. Sci. 2015, 8, 3467–3475.
(22) Woodford, J. J.; Daquin, J. P.; Wilson, K.; Lee, A. F. Better by design: nanoengineered macroporous hydrotalcites for enhanced catalytic biodiesel production. Energy Environ. Sci. 2012, 5, 6145–6150.
(23) Boz, N.; Degirmenbasi, N.; Kalyon, D. M. Conversion of biomass to fuel: Transesterification of vegetable oil to biodiesel using KF loaded nano-particles as catalyst. Appl. Catal., B 2009, 89, 590–596.
(24) Sun, L.-B.; Liu, X.-Q.; Zhou, H.-C. Design and fabrication of nanoengineered macroporous hydrotalcites for enhanced catalytic biodiesel production. Fuel 2012, 95, 1968–1976.
(25) Peng, S.-S.; Lu, J.; Li, T.-T.; Tan, P.; Gu, C.; Wu, Z.-Y.; Liu, X.-Q.; Sun, L.-B. Significant decrease in activation temperature for the generation of strong basicity: a strategy of endowing supports with reducibility. Inorg. Chem. 2019, 58, 8003–8011.
(26) Yoosuk, B.; Udomsap, P.; Puttasawat, B.; Krasae, P. Modification of calcite by hydration-dehydration method for heterogeneous biodiesel production process: the effects of water on properties and activity. Chem. Eng. J. 2010, 162, 135–141.
(27) Harsha Hebbab, H. R.; Math, M. C.; Yafiz, K. V. Optimization and kinetic study of CaO nano-particles catalyzed biodiesel production from Bambaxceiba oil. Energy 2018, 143, 25–34.
(28) Esipovich, A.; Danov, S.; Belousov, A.; Rogozhin, A. Improving methods of CaO transesterification activity. J. Mol. Catal. A: Chem. 2014, 395, 225–233.
(29) Stamenković, O. S.; Todorović, Z. B.; Lazić, M. L.; Veljković, V. B.; Skala, D. U. Kinetics of sunflower oil methanolysis at low temperatures. Bioresour. Technol. 2008, 99, 1131–1140.
(30) Likozar, B.; Levec, J. Effect of process conditions on equilibrium, reaction kinetics and mass transfer for triglyceride transesterification to biodiesel: Experimental and modeling based on fatty acid composition. Fuel Process. Technol. 2014, 122, 30–34.
(31) Arzamendi, G.; Arguinarena, E.; Campo, I.; Zabala, S.; Gandía, L. M. Alkaline and alkaline-earth metals compounds as catalysts for the methanization of sunflower oil. Catal. Today 2008, 133–135, 305–313.
(51) Lukić, I.; Kesić, Z.; Maksimović, S.; Zdujić, M.; Liu, H.; Krstić, J.; Skala, D. Kinetics of sunflower and used vegetable oil methanolysis catalyzed by CaO-ZnO. *Fuel* 2013, 113, 367−378.

(52) Lukić, I.; Kesić, Z.; Zdujić, M.; Skala, D. Calcium diglyceroxide synthesized by mechanochemical treatment, its characterization and application as catalyst for fatty acid methyl esters production. *Fuel* 2016, 165, 159−165.

(53) Wang, L.; Di, C.; Li, T.; Chun, Y.; Xu, Q. Preparation and catalytic behavior of biomorphic calcium oxide/carbon solid base materials. *Catal. Sci. Technol.* 2015, 5, 5185−5195.