



Transesterification of *Nannochloropsis oculata* microalga's lipid to biodiesel on Al₂O₃ supported CaO and MgO catalysts

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ABSTRACT

In this study, we present the activities of Al₂O₃ supported CaO and MgO catalysts in the transesterification of lipid of yellow green microalgae, *Nannochloropsis oculata*, as a function of methanol amount and the CaO and MgO loadings at 50 °C. We found that pure CaO and MgO were not active and CaO/Al₂O₃ catalyst among all the mixed oxide catalysts showed the highest activity. Not only the basic site density but also the basic strength is important to achieve the high biodiesel yield. Biodiesel yield over 80 wt.% CaO/Al₂O₃ catalyst increased to 97.5% from 23% when methanol/lipid molar ratio was 30.

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1. Introduction

The negative impacts of global warming, now accepted as a serious problem by many people, have clearly been observed for past decade and seem to intensify every year. The release of the carbon oxides and related inorganic oxides are more than the amount that could be absorbed by the natural sinks in the world since 88% of the world energy demand is provided by carbon based non-renewable fuels (Baruch, 2008). It is vital to develop solutions to prevent and/or reduce the emission of greenhouse gases, such as carbon dioxide, to the atmosphere. Carbon dioxide neutral fuels like biodiesel could replace fossil fuels.

Biodiesel seem to be a viable choice but its most significant drawback is the cost of crop oils, such as canola oil, that accounts for ~80% of total operating cost, used to produce biodiesel (Demirbas, 2007). Besides, the availability of the oil crop for the biodiesel production is limited (Chisti, 2008). Therefore, it is necessary to find new feedstock suitable for biodiesel production, which does not drain on the edible vegetable oil supply. One alternative to oil crops is the algae because they contain lipids suitable for esterification/transesterification. Among many types of algae, microalgae seem to be promising because:

- (1) They have high growth rates; e.g., doubling in 24 h (Rittmann, 2008).

- (2) Their lipid content could be adjusted through changing growth medium composition (Naik et al., 2006).
- (3) They could be harvested more than once in a year (Schenk et al., 2008).
- (4) Salty or waste water could be used (Schenk et al., 2008).
- (5) Atmospheric carbon dioxide is the carbon source for growth of microalgae (Schenk et al., 2008).
- (6) Biodiesel from algal lipid is non-toxic and highly biodegradable (Schenk et al., 2008).
- (7) Microalgae produce 15–300 times more oil for biodiesel production than traditional crops on an area basis (Chisti, 2007).

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Miao and Wu (2006) reported that it was possible to heterotrophically grow microalgae, *Chlorella protothecoides*; hence resulting in an increase of 55% in the lipid content and also the algal lipid could be transformed into biodiesel with sulfuric acid transesterification under 1:1 weight ratio of acid to lipid and 56:1 molar ratio of methanol to lipid at 30 °C. In addition, Li et al. (2007) showed that it was feasible to grow microalgae, *C. protothecoides*, in a commercial scale bioreactor and also using 75% immobilized lipase, they claimed that 98.15% conversion could be obtained in 12 h when the reaction condition with respect to solvent type, water content, pH was optimized. On the other hand, Hossain et al. (2008) studied biodiesel production from algal oils of macroalgae species *Oedogonium* and *Spirogyra* using sodium hydroxide as the catalyst and reported that it was possible to produce biodiesel using even low lipid containing macroalgae. Although there are few studies focused on biodiesel production from different algae species using homogeneous catalysts, such as NaOH and sulfuric

acid, there are no studies on the biodiesel production from microalgae species using heterogeneous catalysts in the literature.

In this manuscript, biodiesel production from yellow green algae, *Nannochloropsis oculata* (*N. oculata*) (preferred due to its high fatty acid content), over single step sol–gel synthesized alumina supported magnesium oxide and calcium oxide catalysts was studied as a function of CaO and MgO loading and the methanol/lipid ratio at 50 °C.

2. Methods

2.1. Catalyst preparation

Magnesium oxide, MgO, and calcium oxide, CaO, on alumina, Al₂O₃, catalysts were synthesized using a modified single step sol–gel method (Yoldas, 1975). For example, to prepare 80 wt.% CaO on Al₂O₃; first, necessary amounts of aluminum isopropoxide (AIP) (98%, from Fluka Inc.), water and HNO₃ were mixed at 85 °C under total reflux for 1 h. Then, calcium nitrate tetrahydrate (99.3%, from Fluka Inc.) was added. Finally, excess water was removed with slow evaporation to obtain the mixed oxide gel. All the gels were dried at 120 °C for 18 h, and then calcined at 500 °C for 6 h. Catalysts were ground and sieved to 300 mesh and stored in desiccators. The same procedure was applied to synthesize MgO on Al₂O₃ catalyst but magnesium nitrate hexahydrate (98%, from Fluka Inc.) was used as the precursor for MgO. Pure MgO was prepared by precipitating magnesium nitrate at pH of 9 with NH₄OH solution at room temperature and washed with deionized water for several times before applying the same heat treatment procedure used for the gels. Pure CaO (99+%, from Aldrich Inc.) was used as received without any treatment.

2.2. Algal lipid extraction and biodiesel production

N. oculata algal paste was obtained from Dr. Durmaz of Ege University (Durmaz, 2007). The wet paste collected by centrifuging was dried at 60 °C under vacuum of 100 mbar for 12 h. Then, microalgal lipid was extracted with hexane in a Soxhlet extractor operated at 80 °C for 10 h using similar procedure given by Miao and Wu (2006). The procedure used in this study is rather partial extraction because of only non-polar solvent, i.e., hexane, used in the extraction. In fact, total lipid extraction requires methanol–chloroform mixture and using hexane eliminates non-lipid extracts (Hara and Radin, 1978). In the final step, the excess hexane was evaporated using a rotary evaporator at 50 °C and 100 mbar to obtain the lipid. In this study, we used the same microalgae species, the growth medium and the harvesting time as given in the literature (Durmaz, 2007; Fredriksson et al., 2006; Roncarati et al., 2004) but the main difference between this study and the cited references is the type of the extraction solvent. For instance, hexane:isopropanol and chloroform:methanol solvents were generally used in the extraction (Fredriksson et al., 2006; Roncarati et al., 2004). Thus, total lipid extraction was possible as indicated in the cited references. They reported that the total lipid consisted of ~55% fatty acids, ~29% phospholipids and the rest being sterols. However, in this study, hexane was only used in the extractor fitted with 1 µm filter paper; hence, resulting in partial extraction and also the elimination of possible non-soluble contents. The algal lipid obtained after hexane evaporation was used in the heterogeneous transesterification without further treatment. Biodiesel was produced under a constant reaction condition: 50 °C, 2 wt.% of oil used as the catalyst and 1100 rpm of stirring speed. The effect of methanol–lipid ratio was studied using 6:1 and 30:1 methanol to lipid ratios. The reaction time was set to 4 h for all the catalysts based on our previous studies done on canola and sunflower oils using heterogeneous catalysts (Umdu, 2008).

2.3. Catalyst activity and characterization measurement

Biodiesel content was measured by using a Shimadzu GC-17A gas chromatograph equipped with a FID detector and a DB-WAX 122-7032 column with a 30 m of column length, 0.25 mm of column diameter and 0.25 µm of film thickness. The separation was achieved using an isothermal analysis method: column was at 225 °C and for injection port and detector, 250 °C was used. Helium flow and split ratio were set at 32 cm/s and 150, respectively. Weight percentage based biodiesel yield is defined as:

$$\text{Biodiesel yield (wt.\%)} = 100 \times \frac{\text{(amount of biodiesel (g) in upper mixture)}}{\text{(initial amount of microalgal lipid (g))}}$$

Crystallite phases present in the catalysts were determined by X-ray diffraction technique (Philips X'pert Pro XRD, operated at 40 kV and 45 mA) and also average crystallite sizes were calculated from the peak broadening of the diffraction peaks using Scherrer equation. In order to determine the basicity and basic strength of all the catalysts, carbon dioxide temperature programmed desorption (CO₂ TPD) technique was used. The amount of CO₂ irreversibly adsorbed is a good measure of the total amount of basic sites on solid surfaces and also, desorption temperature of CO₂ is the indication of strength of the basic site; for instance, the stronger the basic sites, the higher the temperature (Tanabe et al., 1989). In CO₂ TPD, CO₂ adsorption was first carried out for 1 h at room temperature with 30 mL/min of CO₂ flow over 0.1 g of the catalyst after cleaning the catalyst at 500 °C using helium. This was followed by helium purge at room temperature to remove gas phase and weakly adsorbed CO₂. Desorption was performed under 20 mL/min of helium gas while ramping the temperature from room temperature to 500 °C at 10 °C/min of heating rate.

3. Results and discussion

To the best of our knowledge, there are no studies on the production of biodiesel from the lipids of *N. oculata* using heterogeneous catalysts. Fig. 1 compares the biodiesel yields obtained on all the catalysts under the reaction condition of 50 °C and 6:1 of methanol–lipid ratio. As seen in the figure, the catalysts with 80% loading are the most active catalysts; being 4–5 times more active

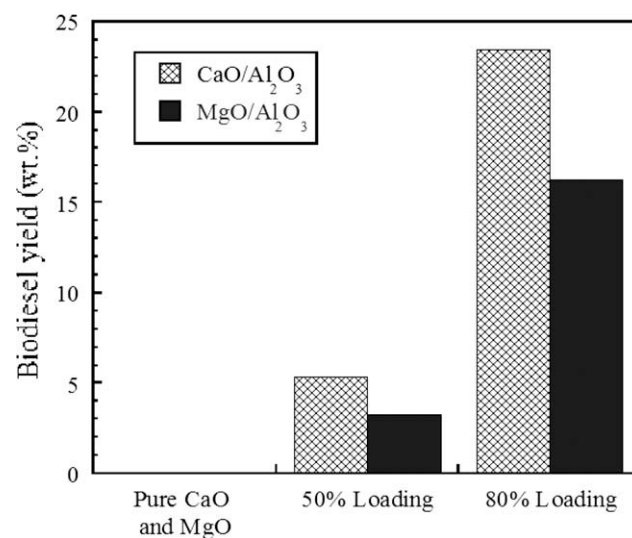


Fig. 1. Biodiesel yield of CaO/Al₂O₃ and MgO/Al₂O₃ catalysts as a function of CaO and MgO loading and pure CaO and MgO. Reaction condition: methanol/oil ratio, 6:1; 50 °C.

than those with 50% loading. For 80 wt.% loading, the biodiesel yield is ~23% over CaO/Al₂O₃ while it is ~16% over MgO/Al₂O₃. Also, we found that pure CaO and MgO catalysts were not active for transesterification of microalgal lipid. Based on these findings, we focused on 80 wt.% CaO/Al₂O₃ catalyst to examine the effect of methanol–lipid ratio on the biodiesel yield and also the reusability of the catalyst under the reaction condition. The reaction temperature, the catalyst amount and the reaction time were kept constant. As the methanol–lipid ratio was increased from 6:1 to 30:1; the biodiesel yield increased from ~23% to 97.5%. This increase indicates that the forward transesterification reaction rate was accelerated with the increase of methanol amount; similar to that observed with vegetable oil transesterification under excess methanol (Umdu, 2008).

We also checked the reusability of 80% CaO/Al₂O₃ catalyst and effects of possible poisons coming from the microalgal lipids, such as phospholipids, on the biodiesel yield. At the end of the reaction (after 4 h), without removing the catalyst from the reactor, we added additional amount of microalgal lipid and necessary amount of methanol and then kept the reactor at 50 °C for additional 4 h. Then, we analyzed the content for biodiesel and in this second time usage; we found the same biodiesel yield as given in Fig. 1 within our experimental error of ~5%. This also proves that there was no coke formation (although at 50 °C, it is not in general expected) or any other adverse effects of possible poisons on the catalyst activity. One should notice that this catalyst was used only twice for a total of more than 10 h of the reaction time. But further tests for long term usages are needed to evaluate for large scale industrial applications.

In the transesterification of oils with a low amount of free fatty acids, the most active catalysts are known to be basic catalysts. Although CaO and MgO are basic solid catalysts, we find that they are not active for the transesterification of the microalgal lipid under the reaction conditions used in this study. However, when they are mixed with Al₂O₃ (also not active for microalgal lipid transesterification), the transesterification activity increases as a function of the amount of CaO and MgO on Al₂O₃. This is interesting because one may ask if the increased activity observed over mixed oxides is due to the dispersion of CaO and MgO crystallites in Al₂O₃ or the creation of new basic sites. To answer this question, XRD and CO₂ TPD techniques were used.

XRD patterns of all the CaO/Al₂O₃ catalysts are shown in Fig. 2. For 50% and 80% CaO catalysts, the small peaks seen in the figure do not correspond to the diffraction pattern of pure CaO crystallite phase. But they were identified as the mixture of calcium aluminate hydrate and/or alumina hydrate formed due to adsorbed

water on the catalysts (JCPDS-ICDD, 2000). This seems to be plausible because the samples were open to atmosphere prior to and during the XRD measurements; hence resulting in the adsorption of water from air. From the peak broadening, the crystallite sizes of CaO and MgO were calculated using Scherrer equation. In CaO based catalysts, the average crystallite size for pure CaO was 164 nm whereas 50 and 80 wt.% CaO/Al₂O₃ catalysts were XRD amorphous with respect to CaO crystallite phase (i.e., no diffraction pattern corresponding to CaO phase) while small peaks related to hydrated calcium aluminate and alumina crystallite phases are present, as seen in Fig. 2. Since the integration of the small peaks was not possible and XRD technique is sensitive the crystallite thickness larger than 5 nm, one could speculate that the average crystallite size of CaO in CaO/Al₂O₃ catalysts must be less than 5 nm and that of hydrated calcium aluminate could be around 5 nm. The high dispersion of CaO on Al₂O₃ found in this study is in parallel with the literature. For example, Cinibulk (1998) showed that CaO could extremely well be dispersed on Al₂O₃ and this was affected by the preparation method (colloidal and polymeric sols) and the precursors of calcium and alumina. Baik and Moon (1991) reported that the presence of CaO inhibited the sintering of Al₂O₃; similar to our findings that there was no clear diffraction pattern corresponding to a specific alumina crystallite phase.

Fig. 3 shows that on all the MgO/Al₂O₃ catalysts, the diffraction pattern of MgO crystallite phase is clearly observed at 2θ angles of ~37°, ~43° and ~62° and also that of γ-Al₂O₃ crystallite phase at ~37°, ~46° and ~67° 2θ angles (JCPDS-ICDD, 2000). Surprisingly, we did not observe significant adsorption of water on MgO catalysts. In fact, Jiang et al. (2001) reported that MgO could be well-dispersed on Al₂O₃ when MgO loading was below 17 wt.% and also, the temperature must be above 570 °C to form Mg–Al spinel; otherwise, its formation was negligible but MgO crystallite size increased with MgO loading. This confirms our findings that there was only MgO and γ-Al₂O₃ crystallite phases on MgO/Al₂O₃ catalysts since MgO loading in all our catalysts were well above 17 wt.% and also we calcined all the catalysts at 500 °C. As seen in Fig. 3, MgO peaks are superimposed on γ-Al₂O₃ peaks at ~37°, ~46° and ~67° of 2θ angles. Using Scherrer equation, we found that the average MgO crystallite size for MgO based catalysts was: 24.9 nm in pure MgO and 24.8 nm in 50% MgO/Al₂O₃ and 20.4 nm in 80% MgO/Al₂O₃. One should notice that there is no significant difference in the average crystallite thicknesses for MgO catalysts, but the biodiesel yields obtained over MgO/Al₂O₃ catalysts increase with MgO loading for MgO/Al₂O₃. Thus, it seems that

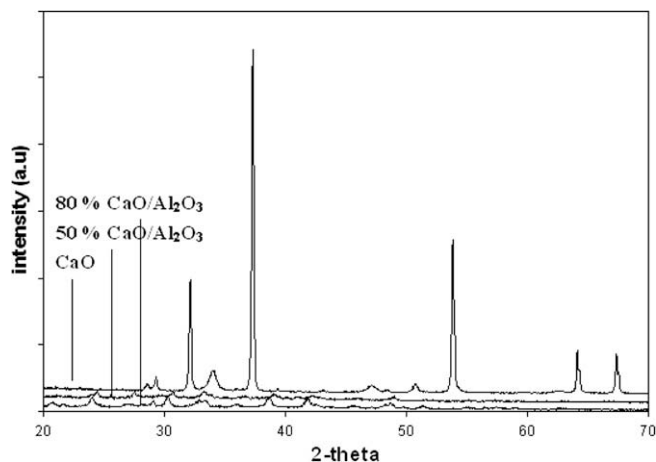


Fig. 2. XRD patterns of CaO based catalysts.

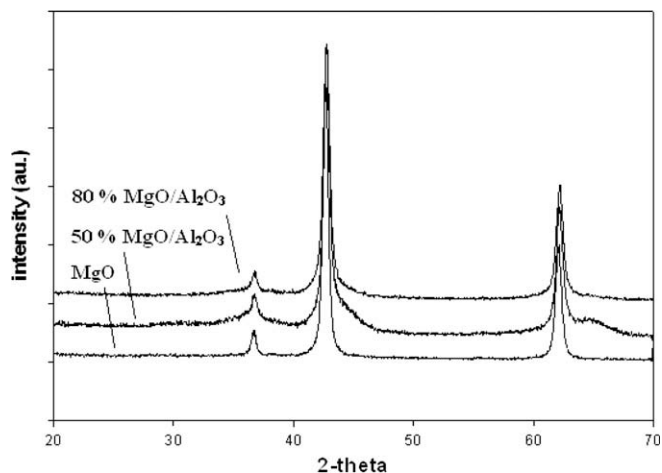


Fig. 3. XRD patterns of MgO based catalysts.

Table 1
Basicity and basic strength of the selected catalysts.

Catalyst	Basicity (μmol of CO_2 per m^2)	Basic strength (peak temperature $^\circ\text{C}$)	Full width at half height ($^\circ\text{C}$)
50% CaO/ Al_2O_3	16.4	300	50
80% CaO/ Al_2O_3	45.5	407	73
50% MgO/ Al_2O_3	27.9	147	99
80% MgO/ Al_2O_3	10.0	180	85
	8.8	472	39

the crystallite size of MgO in MgO/ Al_2O_3 catalysts does not have a direct effect on the biodiesel yield.

The basic site density (the number of basic sites per square meter) of pure CaO and pure MgO is much higher than that of their mixed oxides. CO_2 TPD results as seen in Table 1 show that although total basic site density of 50 wt.% MgO/ Al_2O_3 is almost twice that of 80 wt.% MgO/ Al_2O_3 , the biodiesel yield over 50 wt.% MgO/ Al_2O_3 is much lower than that 80 wt.% MgO catalyst. This inverse trend in biodiesel with total basic site density may be explained by the difference in basic strength of the catalysts. In fact, we found that the basic strength of 50 wt.% MgO/ Al_2O_3 was $\sim 50^\circ\text{C}$ lower and narrower (as indicated by full width at half height) than that of 80 wt.% MgO/ Al_2O_3 . Therefore, it seems that not only the basic site density but also the basic strength is important in the transesterification of microalgal lipid over MgO catalysts. For CaO based catalysts, the total basicity of 50 wt.% CaO/ Al_2O_3 is 33.6 whereas it is 45.5 on 80 wt.% CaO/ Al_2O_3 . Although the basicity of 80 wt.% CaO is $\sim 34\%$ higher than that of 50 wt.% CaO and also they have similar average crystallite sizes ($< 5\text{ nm}$), its transesterification activity is ~ 3.5 times that of 50 wt.% CaO. So, similar to MgO catalysts, this indicates that the basicity alone is not enough to achieve high transesterification activity over CaO catalysts. The basic strength distribution on 50 wt.% CaO is bimodal; one peak centered at 300°C and the another at 488°C but on 80 wt.% CaO, the basic strength distribution is uniform and centered at 407°C . In other words, under similar basic site densities, the basic strength of 80 wt.% CaO catalyst is mild enough (not too high nor too low) to achieve the high biodiesel yield. Our work on the effect of methanol amount, CaO loading and the reaction temperature continues and will be published in near future.

4. Conclusions

In this work, we showed Al_2O_3 supported CaO and MgO catalysts were more active than pure CaO and MgO in the production of biodiesel from the microalgae *Nannochloropsis oculata*. Among the mixed oxide catalysts, 80 wt.% CaO/ Al_2O_3 was the most active and could be reused at least twice. We found that its activity was

not directly related the crystallite sizes of CaO in Al_2O_3 but rather related to the basic site density and basic strength generated during sol-gel synthesis. In fact, the high basic site density and mild basic strength is required for the high biodiesel yield. Excess methanol is necessary to achieve 97.5% biodiesel yield.

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