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Functionalized Fe₃O₄@Silica Core–Shell Nanoparticles as Microalgae Harvester and Catalyst for Biodiesel Production

Harvesting and fuelling: Core–shell Fe₃O₄@silica magnetic nanoparticles functionalized with a strong base, triazabicyclodecene (TBD), were successfully synthesized for harvesting microalgae and for one-pot microalgae-to-fatty acid methyl ester (FAME, or so-called biodiesel) conversion. Covalently functionalized core–shell nanoparticles have a large potential for the production of liquid transportation fuels from algal biomass.
The Inside Cover illustrates how Fe₃O₄@silica core–shell magnetic nanoparticles functionalized with a strong base [i.e., triazabicyclodecene (TBD)] can be used for sequential microalgae harvesting and for the one-pot conversion of microalgae to fatty acid methyl ester (biodiesel). While being effective for transforming algae oil of multiple algae sources, its excellent adsorption and magnetic properties make TBD-Fe₃O₄@silica nanoparticles exceptionally suitable for algae harvesting. A wide range of other covalently functionalized nanoparticles can be envisaged to be applied for the production of liquid transportation fuels from algal biomass. More details can be found in the Communication by Wu et al. on page 789 (DOI: 10.1002/cssc.201402996).
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the catalyst for complex three-component coupling process and attachment of a desired organic functionality can add tremendous advancement. Moreover, for esterification of glycerides obtained from algae oil, a nucleophilic base functionalization of magnetic silica particles was essential.

Subsequently, a strong base, that is, triazabicyclodecene (TBD), was attached to the surface of the FeOx@silica nanoparticles through a covalent bonding of nucleophilic pyrimido-nitrogen of TBD with the epoxy-containing silane (trimethoxysilylpropoxymethylxirane) grafted on the silica surface. This strategy of functionalizing silica surface with the covalent attachment of TBD depends on the grafting of the epoxysilane on silica by interacting with the surface silanol groups. This keeps the epoxy group of attached silane open for further functionalization.

The small particle size of the TBD-functionalized FeOx@silica nanoparticles (denoted as TBD-FeOx@silica NPs) was found to have an interaction with the micron-to-millimeter sized microalgae. Thus, when TBD-FeOx@silica NPs were added to a microalgae suspension, the resulting mixture could be collected and separated using a magnetic stone, which indicated that the synthesized TBD-FeOx@silica NPs could be used as a harvester for microalgae. Subsequently, the mixture was added to a mixed solution containing chloroform and methanol. Such a co-solvent system has been reported to be useful for the extraction of algal lipid from microalgae. Because the co-solvent system contains methanol that is also a reactant for transesterification, the extracted algal lipid would continue to be converted to biodiesel because the synthesized TBD-FeOx@silica NPs act as an effective basic solid catalyst.

A designed core–shell catalyst was constructed by synthesizing FeOx nanoparticles as the cores and subsequently coating a silica layer on the external surface of the FeOx cores (i.e., FeOx@silica NPs). The average sizes of the prepared FeOx and FeOx@silica NPs observed using SEM and TEM (Figure 1a–c, Figure S1) were 15.2 and 20 nm, respectively, which are smaller than the hydrodynamic diameters (33.4 nm and 84.9 nm for FeOx and FeOx@silica NPs, respectively) measured using a Zetasizer (Nano ZS, Malvern Instruments Ltd.; Figure 1d). A large particle-size of FeOx@silica NPs indicates successful silica layer coating and a uniform size distribution, which result from our synthesis strategy. The core–shell structure of FeOx@silica NPs was confirmed from TEM observations (Figure 1c), in which the silica shell thickness can be estimated to be 4.8 nm. The crystalline structure of the FeOx core was confirmed from X-ray diffraction (XRD, Rigaku Ultima-IV) analysis, and the corresponding crystal size calculated using the Scherrer equation was around 16 nm, which was consistent with the value measured using TEM (Figure S2). The porous properties of FeOx@silica NPs were studied from N2 adsorption/desorption isotherms, and the Brunauer–Emmett–Teller (BET) specific surface area was calculated to be 103.4 m²·g⁻¹ (Figure S3). The large external surface area of the synthesized FeOx@silica NPs provides a sufficient number of sites for interaction with microalgae. The superparamagnetism of the synthesized FeOx@silica NPs was confirmed using superconducting quantum interference device analysis. A plot of magnetization versus magnetic field is shown in Figure S4; the S-shaped curve without hysteresis loops indicates the absence of an induced magnetic field in the sample when the external magnetic field was removed.
The functionalization of Fe$_3$O$_4$@silica NPs with TBD was achieved in two steps: silanation of trimethoxysilylpropoxymethylloxirane onto the silica shell, followed by treatment with 1,5,7-triazabicyclo[4.4.0]dec-5-ene. The successful functionalization was confirmed from Fourier transform infrared (FTIR) spectra. A weak C–H bending signal at 1450 cm$^{-1}$ and a C–H stretching signal at 2800 to 3000 cm$^{-1}$ were observed (Figure 2 and Table S1, Supporting Information), corresponding to the carbon backbone of silane and TBD, respectively. Comparing the C–H and C–N (aryl) stretching signals of the samples before and after functionalization, we could confirm that the Fe$_3$O$_4$@silica NPs were indeed functionalized with TBD. TBD was quantified using a titration process, and its amount was estimated to be 1.77 mmol g$^{-1}$. In addition, the surface charges of Fe$_3$O$_4$ NPs, Fe$_3$O$_4$@silica NPs, and TBD-Fe$_3$O$_4$@silica NPs were measured to be $-30$, $-45$, and $-38$ mV, respectively. The decrease in the negative surface charge upon functionalization also indicated the successful addition of TBD groups.

To demonstrate the effective harvesting of microalgae, TBD-Fe$_3$O$_4$@silica NPs were added to a suspension of microalgae, and a magnetic stone was placed at the bottom of the solution. The microalgae/TBD-Fe$_3$O$_4$@silica NP mixture was collected by the magnetic stone (Figure 3) within one minute. Because the size of microalgae is several tens of micrometers, we suggest that TBD-Fe$_3$O$_4$@silica NPs of several tens of nanometers in size would be necessary to attach to the microalgae. Thus, the whole mixture can be easily collected by magnetic stone.

To study the feasibility of using the synthesized TBD-Fe$_3$O$_4$@silica NPs for producing biodiesel from microalgae, three types of algae oil sources, dried algae, algae oil, and algae concentrate (i.e., algae/water mixture), were evaluated. The algae concentrate was produced by removing most of the water from the algae suspension after centrifuging; however, the algae concentrate still contained water. The dried algae were produced after putting the algae concentrate in a lyophilizer and removing all the water. The algae oil was obtained by putting dried algae in a mixed solution of chloroform/methanol (v/v = 1:2) at 65 °C for 18 h in order to break the cell wall and extract algal lipid from wet algae (i.e., co-solvent method). In the case of the algae concentrate, we found that it was not stable upon storage, that is, the composition of FAMEs varied as the storage time (at 4 °C) increased (Figure S5). Therefore, we decided to use the three algae sources immediately after harvesting to avoid errors.

Moreover, in the case of the algae concentrate, we found that the total FAME amount increased when the storage day exceeded 10 days, and the reason was the formation of FFAs. Apart from storage conditions, we also studied the effect of water content on biodiesel production when using algae concentrate as the oil source. We found that the FAME yield decreased considerably when the water content of the algae concentrate exceeded 0.05 wt % (Figure S6). Therefore, the water content of the algae concentrate used in this study was fixed at 0.03 wt %.

The conversion process of microalgae was dependent on the presence/absence of FFAs, when our TBD-Fe$_3$O$_4$@silica NPs were used as the basic catalyst. If the microalgae contained FFAs, then the FFAs were first converted to biodiesel by using "acidic" catalysts to avoid saponification. In such a case, we used different acidic catalysts, including H$_2$SO$_4$, AlCl$_3$, and Amberlyst-15, and compared their esterification efficiencies. The results in Table 1 indicate that H$_2$SO$_4$ showed the highest FAME yield (around 20%). Notably, the FAME yield was low when Amberlyst-15 was used. The reason for this was that the solid acid catalyst could not penetrate through the cell wall and interact with the algal lipid. After esterification of FFAs, we added the TBD-Fe$_3$O$_4$@silica NPs, which were used as a solid base catalyst for the transesterification of algal lipid; consequently, an enhanced FAME yield of 37.8 % was obtained (Table 1).

To demonstrate that the cell wall of the microalgae indeed inhibits the conversion of algal lipid to biodiesel, we compared two different algae sources: dried algae (with cell wall) and algae oil (without cell wall). The algae concentrate was not se-
lected here because we wanted to eliminate the effect of FFAs and to fix the composition of algae oil. Moreover, since the algae oil sources did not contain FFAs, we could directly use either an acid or a base catalyst for one-step transesterification. In the case of dried algae where acid catalysts were used, homogeneous acids (i.e., H$_2$SO$_4$ and AlCl$_3$) gave higher FAMEs yields compared to heterogeneous catalysts (i.e., Amberlyst-15), as shown in Table 2 (Raw 1–3). Similar results were ob-

The existence of the cell wall indeed suppressed the catalytic efficiency of solid catalysts because of the difficulty the catalyst faced in penetrating the cell wall. Several sophisticated techniques for cell-wall disruption have been developed for efficient extraction of intercellular lipids,[11] and in this study, we chose the co-solvent method to obtain algae oil in order to test the performance of the synthesized solid catalysts. A high FAME yield could be obtained through the transesterification of algae oil, indicating the excellent conversion efficiency of the strong base group (i.e., TBD-containing silica shell).

In summary, the efficient production of biodiesel through the transesterification of algae oil was achieved by using highly basic TBD-Fe$_3$O$_4$@silica core/shell nanoparticles containing a magnetic core and a catalytic shell. The magnetic core helps collect the microalgae. After the elimination of FFAs and extraction of algae oil from the microalgae, the catalytic shell converted the algae oil to biodiesel with a high yield (max. yield: 97.1%). This study heralds the emergence of a new area of functionalized core–shell nanoparticles that can be used for the production of biodiesel and other liquid fuels from algal biomass.

### Experimental Section

**Chemicals:** 1,5,7-Triazabicyclo[4.4.0]dec-5-ene, 2,2-dimethoxypropane, aluminum chloride, Amberlyst-15, copper chloride, sulfuric acid, tetraethoxysilane (TEOS), and trimethoxysilylpropanethoxyloriane were purchased from Sigma–Aldrich (Taipei, Taiwan), Hep-

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<th>Table 1. Two-steps conversion of algal biomass to biodiesel.</th>
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<th>Table 2. One-step conversion of algal biomass to biodiesel.</th>
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<td>Oil sources (without FFAs)</td>
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Fe$_3$O$_4$@silica NP catalyst. This yield was higher than those for strong homogeneous (H$_2$SO$_4$) and heterogeneous (Amberlyst-15) as acid catalysts. These results indicate that the synthesized base catalyst TBD-Fe$_3$O$_4$@silica NPs exhibited greater catalytic performance compared to acid catalysts for the one-pot transesterification of algae oil to biodiesel.

As we mentioned earlier, the microalgae-to-biodiesel conversion involves three important tasks: harvesting, cell wall disruption, and transesterification. The development of a new technique for harvesting microalgae is the first priority. To date, apart from the development of numerous techniques for microalgae harvesting, only a limited number of nanomaterials have been identified for efficient sedimentation (harvesting) of microalgae with a short time frame.[12] In this study, the synthesized TBD-Fe$_3$O$_4$@silica NPs were found to show outstanding algae harvesting features because of their magnetism (Fe$_3$O$_4$ core). After harvesting the microalgae, the compositions of algae oil and FFAs varied upon storage if the water content was very high (i.e., if the algae concentrate had a water content exceeding 0.05 wt %). The presence or absence of FFAs in the microalgae determined whether the two-step esterification-transesterification process or the one-step transesterification process was to be used, respectively, and the appropriate catalysts were used.

- **Chemicals:** 1,5,7-Triazabicyclo[4.4.0]dec-5-ene, 2,2-dimethoxypropane, aluminum chloride, Amberlyst-15, copper chloride, sulfuric acid, tetraethoxysilane (TEOS), and trimethoxysilylpropanethoxyloriane were purchased from Sigma–Aldrich (Taipei, Taiwan), Hep-
tane, toluene, and hexane were purchased from J. T. Baker. Iron(III) chloride hexahydrate (FeCl₃·6H₂O) was purchased from Alfa Aesar. All chemicals and solvents were used without any further purification.

**Synthesis of TBD-functionalized Fe₃O₄@silica nanoparticles:** The method used for the preparation of Fe₃O₄ nanoparticles was a modified version of a method used in a previous procedure.[16] Typically, sodium alginate (100 mg) was dissolved in 750 mL water under vigorous stirring for 30 min, and subsequently, 0.97 g of ferrous chloride tetrahydrate (FeCl₂·4H₂O) and 1.68 g of ferric chloride hexahydrate (FeCl₃·6H₂O) were added. A clear solution was obtained after stirring for another 30 min. The formation of Fe₃O₄ was induced by the drop wise addition of aqueous ammonia solution, which increased the pH from 2.2 to 10. Finally, Fe₃O₄ nanoparticles were collected using a magnetic stone, washed with water and methanol repeatedly to remove excess ammonia, and stored in absolute ethanol to prevent oxidation of samples.

The method used for coating a silica layer on the Fe₃O₄ nanoparticles was a modified version of a previously reported method.[17] Typically, the synthesized Fe₃O₄ nanoparticles were suspended in absolute ethanol (1.25 mg Fe₃O₄/mL ethanol). Subsequently, 0.8 mL of 28% aqueous ammonia solution and 2 mL of water were added to 20 mL of Fe₃O₄ in ethanol, and this was followed by sonication for 5 min. A magnetic stir bar was placed into the reaction vial just before starting the slow addition of tetraethyl orthosilicate (TEOS, Si(OCH₃)₄), and the resulting Fe₃O₄/silica sample was separated using magnetic force. The sample was then washed with ethanol and water several times to remove excess TEOS, ammonia, and non-Fe₃O₄ containing silica. The samples were dried under vacuum overnight.

The method used for the functionalization of the Fe₃O₄@silica nanoparticles with TBD was a modified method version of a method presented in a previous paper.[18] As shown in Figure 57, first trimethoxysilylpropoxymethyloxirane (i.e., an epoxy-containing silane) was grafted onto the silica surface as follows: Toluene (30 mL) was added to a pre-degassed round bottom flask preloaded with dried Fe₃O₄@silica powder (0.5 g) under N₂, and this was followed by the addition of trimethoxysilylpropoxymethyloxirane (2 mL). The resulting mixture was refluxed at 110 °C for 24 h, and the product was washed with toluene several times before being dried under vacuum. Next, 0.4 g of the dried sample and 0.3 g of 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) were suspended in 15 mL of toluene, and the solution was stirred for 8 h at room temperature. The sample was collected and washed with toluene several times to remove the excess TBD, and dried under vacuum overnight.

**Characterization:** The morphology and core/shell structure of Fe₃O₄@silica nanoparticles were observed using SEM (Nano Nova SEM) and TEM (JEOL JEM 1200EX-II). The porous properties were analyzed using nitrogen adsorption/desorption isotherms on a Micromeritics ASAP 2010 instrument. The specific surface area was calculated using the Brunauer–Emmett–Teller method. The crystallinity of Fe₃O₄ was examined using XRD. The hydrodynamic diameters of the samples were determined with a Zetasizer. The presence of functional groups on the samples was confirmed using FTIR spectroscopy.

**Preparation of algae concentrate, dried algae, and algae oil:** The microalgae (Chlorella vulgaris) was cultured in a neutral medium, and the detailed culture conditions have been described in our previous paper.[19] After centrifugation and washing with deionized water three times, we added various amounts of deionized water to the microalgae to prepare an algae/water mixture (i.e., algae concentrate). Dried algae were produced after putting the algae concentrate in a lyophilizer and removing all the water. Algae oil was produced by putting dried algae in a mixed solution of chloroform/methanol (v/v=1:2) at 65 °C for 18 h (i.e., co-solvent method); this method has been widely adopted for breaking the cell wall and extracting algal lipid.[16] Algae oil was extracted in several batches, and the amount of algae oil extracted was around 29–31 wt% based on the weight of the dried algae. The total amount of oil in algal biomass was determined using the one-step extraction and FAME production method that has been previously reported.[20] Typically, dried algae (200 mg) was added to a mixture containing methanol (1.95 mL), toluene (1 mL), 2,2-dimethoxypropane (0.25 mL), and sulfuric acid (0.1 mL). To simultaneously extract FAMES from the solution, we added heptane (1.7 mL), and the reaction was conducted at 80 °C in an oil bath for 2 h with vigorous stirring. The FAMES in the oil phase were then analyzed using gas chromatography (GC). As shown in Figure S8 and Table S2, the yields from dried algae and algae oil were almost the same with each other (i.e., around 11 wt%). After comparison with a standard sample of FAME containing 37 types of components, we concluded that the major components were from C16:0, C18:0, C18:1, C18:2, and C18:3. The FAME content accounts for approximately one-third of the algae oil, and this is because the algae oil contains biological organs, pigments and other impurities that can be dissolved in the co-solvent, as shown in Figure S9.

**Conversion of algal biomass to biodiesel:** The reaction conditions are summarized in Tables S3 and S4. The catalyst (either a homogeneous or heterogeneous catalyst) was added to methanol solution prior to the addition of algae sources. The system was then sealed to prevent any leakage of the solvent. The entire system was then heated at 65 °C. After reaction for a specific period of time, the solution was pipetted into a centrifugal tube and hexane was added to extract the oil-soluble components from the methanol phase. To achieve a better extraction result, we used a mixer for 1 min and then performed the centrifugation at 5500 rpm for 3 min. The entire hexane phase (upper phase) was extracted to another vial. We extracted products repeatedly until the upper phase turned transparent from yellow. Hexane was then dried in a rotary vaporizer, and the extract was diluted with isopropanol to obtain a total volume of 1 mL. For the removal of the heterogeneous catalysts without magnetic property (e.g., Amberlyst-15), the system was centrifuged at 5500 rpm for 5 min. The FAMES in the oil phase were then analyzed using GC.

**Analytics (compositions of algae oil (reactant) and FAMES (product)):** The analysis of the composition of the produced FAMES was performed using a gas chromatograph (HP-6890) equipped with a flame-ionization detector. Detailed condition settings are summarized in Table S5. The guard column was loaded before the analysis column to trap non-gas substances. The calibration curves of the FAME samples (methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and arachidic acid methyl ester) dissolved in isopropanol were used for calculating the concentration of FAMES. The yields of FAMES were calculated using Equations (1) and (2).

The suffix Cij represents the carbon number of the specific FAME, and the total yields of biodiesel (γy) were determined by summing $\gamma_{y}$ from FAMES of C16 to C19.

\[
\gamma_{y} = \frac{\text{Weight of specific FAME}}{\text{Weight of specific FAME extracted by co-solvent method}}
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\gamma_{y} = \sum_{C_{16}}^{C_{19}} \sum_{k}^{18} \gamma_{ij}
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Acknowledgements

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