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Triflate-catalyzed (trans)esterification of lipids within carbonized algal biomass

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ABSTRACT

This study demonstrates the utility of rare-earth metal triflate catalysts (i.e., $Sc(OTf)_3$ and $In(OTf)_3$) in the (trans)esterification of oleic acid as well as the lipids contained within carbonized algal biomass using ethanol in the presence of water. Both catalysts are highly active between 200 and 235 °C with an ethanol:fatty acid (EtOH:FA) molar ratio of 10–20:1 and showed a high tolerance for moisture. Lipids within hydrochars produced by reacting *Chlorella protothecoides* paste (25% solids) in high temperature water (220–250 °C) were successfully converted into fatty acid ethyl esters (FAEE). The highest FAEE yields (85–98%) were obtained when hydrochars were reacted for 60 min at 215 °C with about 11–13 mol% $Sc(OTf)_3$, a 17–19:1 EtOH:FA molar ratio, and without water. FAEE yields remained as high as 93% in the presence of 9 wt.% water. Our preliminary results warrant further work to optimize triflate-catalyzed in situ (trans)esterification at low catalyst and ethanol loadings.

1. Introduction

Interest has grown in using microalgae as a biodiesel feedstock, largely due to its high lipid productivity relative to terrestrial plants, the opportunity to capture waste carbon dioxide, and the likelihood that its cultivation will not compete with land and water resources that could otherwise produce food for human and animal consumption. Although intriguing in principle, microalgae present a unique challenge in practice: when grown phototrophically, their cultivation typically requires large amounts of fertilizer, carbon dioxide, and fresh water (Pate et al., 2011). Moreover, biomass density within cultivation systems is typically on the order of 1 g L^{-1}, necessitating the use of energy and capital-intensive dewatering methods and complicating the conversion of algal biomass into liquid fuels (Davis et al., 2011; Grima et al., 2003).

Recently, we reported on a potentially lower cost and environmentally preferable process to convert wet algal biomass into biodiesel using hydrothermal carbonization (HTC) and uncatalyzed, supercritical in situ (trans)esterification (IST/E) with ethanol (Levine et al., 2010). In this process, algae are grown sequentially in the light and then in the dark to generate a dense, lipid-rich biomass (10–20% solids; 30–60% total lipids on a dry weight basis). This paste is then reacted in and with hot liquid water (220–250 °C) at autogenic pressures to hydrolyze intracellular lipids, conglomerate cells into an easily filterable solid that retains the lipids (i.e., carbonized solids or hydrochar), and produce a sterile, nutrient-rich aqueous phase. Wet hydrochars recovered by filtration typically contain between 15% and 45% moisture and retain greater than 85% of the total fatty acids (FAs) originally present in the biomass. We previously demonstrated that the lipids within the hydrochar could be converted into biodiesel without prior solvent extraction by use of supercritical ethanol ($T \geqslant 240.9$ °C, $P \geqslant 6.1$ MPa). Here we report on an alternative method of in situ (trans)esterification employing rare-earth metal triflate catalysts at subcritical temperatures and in the presence of water.

Lower rare-earth metal triflate (trifluoromethanesulfonate, $Tf = SO_2CF_3$) catalysts (e.g., $Sc(OTf)_3$) are Lewis acids known for being highly active, inexpensive, non-toxic, reusable, and insensitive to air, moisture, and common heteroatoms (e.g., N, P, S) (Kobayashi et al., 2002; Savage and Rebacz, 2010). Compared to traditional Lewis acid catalysts (e.g., $AlCl_3$), which decompose when exposed to water and are usually required in stoichiometric amounts, and common heterogeneous acid catalysts (e.g., heteropolyacids), which tend to be expensive and rarely outperform conventional methods employing H_2SO_4 (Stamenković et al., 2011; Yan et al., 2010), metal triflates may be a promising alternative for biodiesel production. Although metal triflates have been extensively used in organic synthesis, including the esterification of carboxylic acids with alcohols (Ishihara et al., 1996), only one report in the literature indicates their use in (trans)esterification reactions to produce fatty acid methyl esters (FAMEs) (Socha and Sello, 2010). In this report, transesterification of pure triglycerides (TGs) was 82–99% complete after 20–30 min at 150 °C in a microwave reactor (0.2–2.0 mL, stirred) with 10 mol% $Sc(OTf)_3$ or $Bi(OTf)_3$ and a 48-fold molar excess of methanol. FA esterification was nearly complete in 1 min under the same conditions with just

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1 mol% catalyst. In both cases, FAME yields increased significantly with alcohol loading (from 6:1 to 48:1 methanol:FA ratio), and $Sc(OTf)_3$ and $Bi(OTf)_3$ showed similar activity. Catalyst recovery from esterification reactions was reportedly ~80% with no significant loss in activity during six sequential reactions.

This initial literature report shows that metal triflate catalysts offer a potential pathway to fast reaction rates at conditions milder than those used in the uncatalyzed, supercritical IST/E approach. It is this potential that motivates our interest in these catalysts. While related work in our lab recently demonstrated that several different metal triflates are effective catalysts for making and breaking C—O bonds in an aqueous-phase medium at ~200 °C (Rebacz and Savage, 2010), no previous work has explored the efficacy of these catalysts for biodiesel production in the presence of water. In addition, the literature contains no reports on the use of these catalysts with ethanol rather than methanol. Although methanol is usually less expensive, we chose to work with ethanol because it is less toxic and renewably derived, has superior dissolving power for oils, and produces biodiesel with improved fuel properties (Stamenković et al., 2011). Finally, metal triflates have never been employed with lipid-rich biosolids produced from real biomass nor with the lower alcohol:FA ratios that would be desired for a viable commercial process. While IST/E with homogeneous acid catalysts (e.g., H_2SO_4) has been proposed as a way to obviate traditional solvent extraction by completing oil extraction and transesterification in one-step, the reaction has only proven successful with very high molar ratios of alcohol to FA and is severely inhibited by water (Ehimen et al., 2010; Wahlen et al., 2011). The potential benefits of triflate catalysts for biodiesel production from high FA and high moisture feedstocks together with our previous green chemistry research with these compounds leads us to hypothesize that these catalysts might lead to a transformative technology for biodiesel production.

2. Methods

2.1. Feedstock production

Chlorella protothecoides (UTEX #255) was grown as a biodiesel feedstock using sequential photo- and heterotrophic growth stages. A modified basal medium (Xiong et al., 2008) was used for shake flasks cultured in the light (concentration, mg L^{-1}): KH_2PO_4 (600), K_2HPO_4 (400), $MgSO_4 \cdot 7H_2O$ (400), glycine (100), vitamin B1 (0.03), and $FeSO_4 \cdot 7H_2O$ (1). Flasks also contained A5 trace mineral solution (1.5 mL L^{-1} total volume) and yeast extract (4 g L^{-1}). All media chemicals were used as supplied by Fisher Scientific. Exponentially growing phototrophic cultures were used to inoculate heterotrophic shake flasks containing the same basal medium, except with 0.06 mg L^{-1} vitamin B1, 0.5 mg L^{-1} $FeSO_4 \cdot 7H_2O$, and 30 g L^{-1} glucose. After 3 d incubation in the dark at 28 °C with shaking at 250 rpm, 0.8 L of combined shake flask culture was used to inoculate a 5 L bioreactor (Bioflo3000, New Brunswick) containing 3 L of the heterotrophic media. Glucose was fed intermittently and pH was controlled at 6.4 for 5 d until biomass was harvested by centrifugation (3000 rcf for 10 min) and immediately reacted.

2.2. Hydrothermal carbonization

All high temperature reactions with wet algal biomass were carried out in 316 stainless steel (SS) reactors fashioned from Swagelok parts (2 caps and 1 port connector). For the purpose of generating carbonized solids to be used in (trans)esterification reactions, 28 mL reactors (approximately 18 cm long × 1.4 cm diameter) were loaded with about 21 g of paste. Once loaded, reactors were immersed in a preheated, isothermal fluidized sand bath at 220–250 °C for 60–120 min and then promptly removed and cooled in water. Upon cooling, the aqueous phase and solids were separated by filtering under light vacuum (pre-dried, pre-weighed P2 Glass microfiber filter, Whatman). No additional water was used to rinse the reactor housing. The wet hydrochar was then either reacted directly, stored at 4 °C prior to reaction, or dried (65 °C for 24 h) and then reacted.

2.3. Triflate-catalyzed in situ (trans)esterification

Pure oleic acid and hydrochars were reacted with ethanol in the presence of varying amounts of metal triflate catalysts (i.e., $Sc(OTf)_3$ and $In(OTf)_3$) and H_2O. All catalysts and pure lipids were purchased from Sigma Aldrich and Nu-Check Prep, Inc., respectively, and used as received. Reactions were performed to identify the effects of reaction temperature, reaction time, catalyst type and loading, ethanol loading, water content, and stirring on the molar yield of FAEEs (defined as moles of ethyl ester formed per mol of FA initially present). Reactants were loaded into SS Swagelok reactors (0.6 mL total volume) at the desired proportions and quantities such that the reactors were 85% full at reaction temperatures. The mass of each reactant was recorded to the nearest 0.01 mg (XS205DU, Mettler Toledo). In stirred reactors, Teflon-coated micro stir bars were included (2 mm long × 2.5 mm diameter, Fisher). Once loaded, reactors were submersed in either an isothermal fluidized sand bath (unstirred reactions only) or within 16 mm diameter openings in an aluminum heating block (Dynabloc, ACE Glass) placed atop a stirring hot plate. Both heating elements were controlled by thermocouples inserted into modified reactors loaded with water. Heat up times were on the order of 2 and 10 min for the sand bath and DynaBloc, respectively.

After cooling to room temperature, reactors were unloaded and the reaction mixture was prepared for GC-FID analysis. Hexadecane was used in initial reactions as an internal standard to control for potential losses introduced during reactor unloading. In these reactions, 5–30 mg of hexadecane were loaded along with the reactants, and 6 mL of *n*-heptane and 1 mL of 1 N H_2SO_4 were used to recover and dilute the reaction mixture. Product yields were corrected for hexadecane recovery, which typically ranged from 85% to 98%. Reactions in which the hexadecane recovery was below 85% were excluded from further analysis. In an effort to eliminate possible unintended effects caused by hexadecane acting as a co-solvent in the reaction, as well as to simplify reactor unloading by using a one-phase solvent system, reactions were carried out that contained no internal standard in the reactor and utilized 10–20 mL of ethanol (containing ~6000 mg L^{-1} hexadecane) for product recovery and dilution. Control experiments with mixtures of ethyl oleate, oleic acid, triflate catalyst, ethanol, and water loaded into reactors placed only at room temperature demonstrated recoveries of ethyl oleate and oleic acid exceeding 90%. In both reactor unloading procedures, the diluted reaction mixture was centrifuged to remove fine particles (3000 rcf for 10 min) and then analyzed by GC-FID to determine the amount of FAEE and FA.

Samples were injected (1 μL; 10:1 to 250:1 pulsed split ratio; 260 °C inlet temperature) onto an HP-InnoWax column (30 m × 0.32 mm × 0.25 μm, J&W 1909BD-113) with an initial oven temperature of 150 °C. After a 3 min hold, the temperature was ramped at 6 °C min^{-1} to 260 °C. Helium was the carrier gas at a constant flow rate of 1.0 mL min^{-1}. FID detector temperature was 300 °C and N_2 served as the make-up gas. The retention time of FAMES, FAEE, and FA were identified from standard mixes and peak areas were converted into molar amounts using linear calibration curves composed of at least four points. Molar yields of FAEE were calculated based on the mass of pure FA loaded or