# Chemocatalytic Upgrading of Tailored Fermentation Products Toward Biodiesel

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Biological and chemocatalytic processes are tailored in order to maximize the production of sustainable biodiesel from lignocellulosic sugar. Thus, the combination of hydrotalcite-supported copper(II) and palladium(0) catalysts with a modification of the fermentation from acetone–butanol–ethanol to iso-propanol–butanol–ethanol predictably produces higher concentrations of diesel-range components in the alkylation reaction.

Efficient routes for the synthesis of bio-based liquid fuels have gained considerable attention both commercially and scientifically for the sustainable production of renewable fuels for transportation.[1–5] Nonetheless, the high oxygen content in the biomass impedes its direct conversion to fuel molecules. The processes involved in converting lignocellulosic biomass to higher molecular-weight compounds can be broadly classified as (i) thermochemical (e.g., gasification of biomass to produce syngas, aqueous phase reforming of sugars, and hydrolysis of biomass to produce sugars)[6] or (ii) biological (e.g., fermentative and non-fermentative pathways to produce small chain alcohols).[7–9] There are advantages and disadvantages associated with the aforementioned routes. Because sugars obtained from biomass are highly functionalized molecules, their direct chemical conversion to higher molecular-weight compounds represents a challenging task. Although lignocellulosic sugars can be converted by microorganisms such as E. coli and Sac-charomyces cerevisiae to smaller chain alcohols for blending with gasoline,[7–9] production of longer chain alcohols for jet and diesel blendstocks suffers from low titers and yields due to their inherent toxicity to the microbial hosts.[10, 11]

We recently reported a chemical catalysis route to generate aliphatic ketones (C5–C19) that are components of gasoline, jet, and diesel fuel from the acetone–butanol–ethanol (ABE) produced from a variety of sugar feedstocks by a Clostridial fermentation.[12] In this process, the major pathway involves combining acetone with up to two molecules of alcohol to produce aliphatic ketones up to C11 (Scheme 1). However, C11 and lower carbon compounds only constitute a minor fraction of diesel fuel. Hence, it would be highly desirable to develop a process wherein high concentration of C12+ (carbon length > C11) compounds could be formed in a single operation. Herein, we demonstrate that a significant increase in the selectivity for diesel range components (C12+) can be achieved by integrating improvements in the chemical catalysis with a tailored biomass-derived fermentation mixture of iso-propanol–butanol–ethanol (IBE).

In order to access greater quantities of diesel range hydrocarbons, we envisioned an alternative pathway in which the alcohols would undergo condensation (Guerbet reaction) prior to combining with acetone. However, this strategy was severely impeded by the preference for acetone alkylation over the Guerbet reaction pathway using the previously reported Pd/C-K₃PO₄.[12] This limitation prompted us to investigate the ABE condensation reaction over hydrotalcite-supported metal catalysts, the recyclability of which has been demonstrated in oxidation and condensation reactions.[13–16] Table 1 summarizes the optimization studies on the ABE alkylation reaction. Treatment of ABE mixture (2.3:3.7:1) in toluene with catalytic Ru–HT in the absence of external base at 200 °C provided the desired aliphatic ketones in 20 % yield (entry 1). We envisioned that replacing ruthenium with palladium(0) would increase the efficiency of the ABE alkylation reaction based on our previous studies.[12] Although switching to Pd–HT resulted in modest improvements (entry 1 versus entry 2), replacing toluene with butanol as the solvent increased the overall yield of aliphatic ketones to 58 % (entry 3). The higher yield employing butanol as the solvent was consistent with our previous studies in which butanol demonstrated a high turnover number in the ABE alkylation reaction.[12] Hence, we decided to replace toluene with the biologically-derived butanol in our subsequent optimization studies. A complete conversion of acetone was observed when the reaction temperature was increased to 240 °C, which

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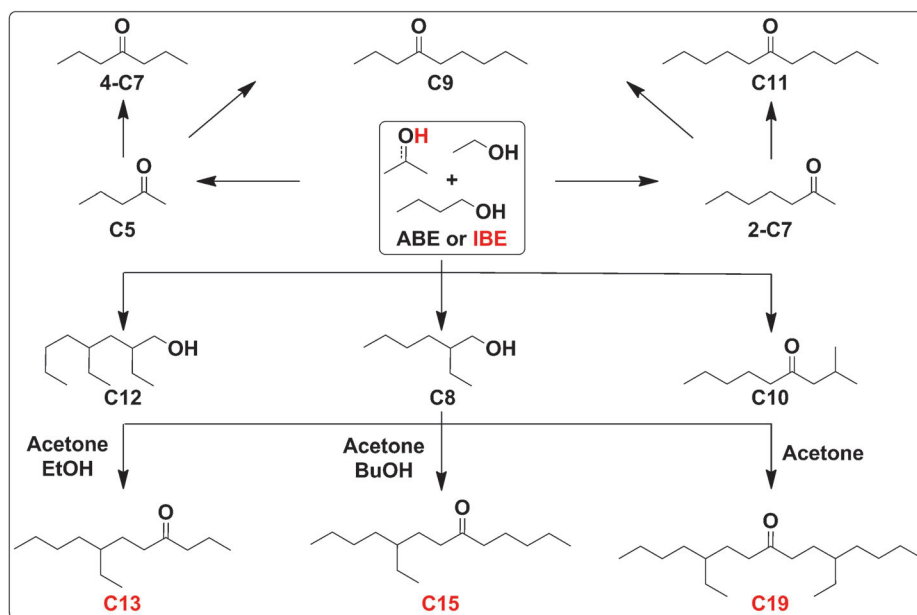
**Scheme 1.** Synthetic Strategy to Upgrade ABE/IBE to Biofuels. C5–C11: gasoline and jet fuels, C13–C19: Diesel

**Table 1.** Optimization of ABE reaction using metal/hydrotalcite catalyst (see Scheme 1).

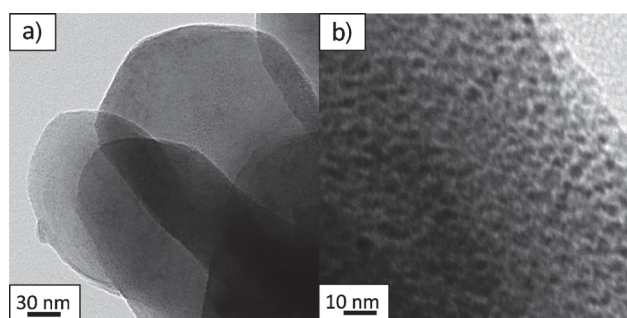| Entry[a] | Catalyst [mol %] | T [°C] | C5 [%] | 4-C7 [%] | 2-C7 [%] | C9 [%] | C10 [%] | C11 [%] | Alcohols[c] [%] | Yield [%] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ru–HT (0.9)[b] | 200 | 0.4 | 2.2 | 12.9 | 0.6 | 0.2 | 3.4 | 0 | 20 |
| 2 | Pd–HT (0.9)[b] | 200 | 0.1 | 1.3 | 17.6 | 0.6 | 0 | 11.9 | 0 | 32 |
| 3 | Pd–HT (0.9) | 200 | 0 | 0 | 39.6 | 1.8 | 0 | 16.8 | 0 | 58 |
| 4 | Pd–HT (1.7) | 240 | 12.1 | 4.4 | 38.2 | 7.3 | 1.6 | 29.5 | 2.0 | 95 |
| 5 | Fe–HT (0.8) | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | Co–HT (0.8) | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | Ni–HT (0.8) | 240 | 0 | 0 | 2.0 | 0 | 0 | 0 | 0 | 2 |
| 8 | Cu–HT (0.8) | 240 | 1.5 | 4.4 | 31.4 | 2.4 | 0.1 | 28.4 | 23.8 | 92 |
| 9 | Zn–HT (0.8) | 240 | 0 | 0 | 2.0 | 0 | 0 | 0 | 0 | 2 |

Reaction conditions: [a] acetone (2.3 mmol), butanol (2 mL), ethanol (1 mmol), 24 h, catalyst loading is represented as the molar percentages with respect to total alcohol loading (ethanol + 1-butanol). Yield based on acetone determined by calibrated internal standard (*n*-dodecane). [b] Acetone (2.3 mmol), butanol (3.7 mmol), ethanol (1 mmol), toluene (1 mL). [c] Alcohols yield (%) for entry 4: 2-C7-OH: 2%; entry 5: 2-C7-OH: 13.4; 4-C7-OH: 9.7; C9-OH: 0.4; and C11-OH: 0.3.

to 0.5 wt% water with respect to the total weight of ABE, which was significantly higher than the previously employed Pd/C-K$_3$PO$_4$ mixture.[12] Further increasing the water content slowed the ABE condensation reaction and resulted in a decreased yield of di-alkylated ketones and a lower overall yield (see Supporting Information, Figure 1). Additionally, the recyclability of both Cu–HT and Pd–HT was investigated by adding fresh ABE to the dried catalyst obtained by centrifugation and phase separation of the supernatant. While Pd–HT could be recycled three times without significant loss of catalytic activity, Cu–HT showed a low degree of deactivation. However, both Pd- and Cu-based hydrotalcite catalysts showed an improved recyclability compared to the first generation Pd/C-K$_3$PO$_4$ which rapidly deactivated in the recycling studies (see Supporting Information, Table 1, Table 2, and Table 3).

Catalyst characterization was carried out in order to evaluate the activity of Cu–HT in the ABE alkylation reaction. The Cu–HT was prepared by adding hydrotalcite (Mg$^6$Al$_2$(OH)$_{16}$CO$_3$·4H$_2$O) to an aqueous solution of copper(II) acetate at 60°C. High-resolution transmission electron microscope (HR-TEM) images of Cu–HT catalyst indicated the formation of Cu clusters 2 ± 1 nm in diameter loaded on 150 ±

also mirrored the excellent yield for aliphatic ketones (entry 4). It is highly desirable to replace palladium with an inexpensive and abundant transition metal from an economic standpoint. To this end, we sought to substitute palladium with a first-row transition metal on the heterogeneous support in the ABE reaction. While iron, cobalt, nickel and zinc showed very low activity in the ABE reaction (entries 5, 6, 7 and 9) Cu–HT gratifyingly provided a mixture of aliphatic ketones along with the corresponding mixture of reduced alcohols[17] in 92% overall yield (entry 4).

The conversion of monoalkylated ketones (C5 and C7) to the dialkylated ketones and the recyclability of catalyst in the alkylation reaction necessitate a catalyst that is fairly tolerant to the water formed in the reaction. A water-inhibition study on Cu–HT revealed that the alkylation reaction could tolerate up



**Figure 1.** a) HR-TEM images of 3.2 wt% Cu–HT. b) Higher magnification image of Cu–HT catalyst reveals the size and distribution of the Cu nanoparticles.

50 nm HT support (Figure 1). The clusters were analyzed by extended X-ray absorption fine structure (EXAFS) and X-ray photoelectron spectroscopy (XPS). The X-ray absorption measurements indicated that CuO is the main species in the heterogeneous Cu–HT catalyst (Figure 2). Similar results were obtained by X-ray absorption spectroscopy (XAS) measurements (see Supporting Information, Figure 2 and Figure 3). In situ EXAFS
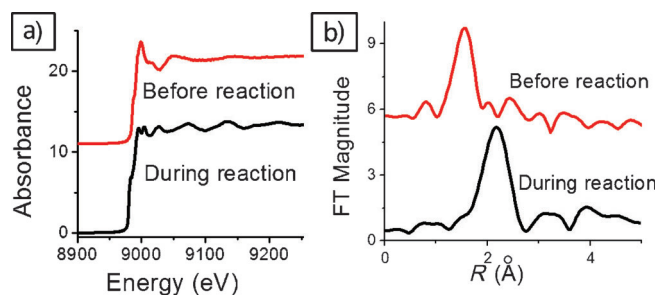


**Figure 2.** a) EXAFS b) and Fourier Transform of EXAFS data of Cu–HT catalyst before (red) and during (black) the catalytic reaction. The shift in the peak position of the FT-EXAFS spectra indicates the reduction of Cu oxide to Cu under reaction conditions.

measurements showed that CuO was reduced to $Cu^0$ at 250 °C under continuous flow of the ABE mixture, prior to the formation of products (Figure 2). These results indicate that the metallic copper nanoparticles are the active catalytic species involved in the dehydrogenation of alcohols to produce aldehydes, which undergo aldol condensation with acetone.

From the outset, we recognized it would be attractive to produce C12+ (C13, C15, and C19) products in the alkylation reaction of fermentative mixture without having to establish an alternate process for diesel components. The C12+ aliphatic ketones are produced from the alkylation reaction of 2-ethyl hexanal with acetone or monoalkylated ketones formed in the reaction. Therefore, we investigated the coupling reaction of acetone with 1-butanol and 2-ethyl hexanol employing Cu–HT at 240 °C, which furnished C11–C19 ketones in 80% overall yield (see Supporting Information). Despite the efficiency of this process, the production of C12+ ketones directly from ABE was still hampered by the competitive direct reaction of the alcohols with acetone.

Though we had been unable to achieve a significant enhancement in the production of C12+ compounds by tuning the catalysts alone, the improved efficiency of the catalytic process allowed us to consider the possibility of in situ formation of acetone by dehydrogenation of isopropanol. We envisioned that the slow production of acetone would potentially permit an increase in the rate of dimerization of butanol to 2-ethyl hexanal (C8) and effectively increase the selectivity toward diesel range components in the product mixture. As predicted, under the optimal condition for

the IBE alkylation reaction, C5–C11 aliphatic ketones were produced in 61% overall yield along with 25 wt% of C12+ compounds (mainly C13 and C15) in the product mixture, a twofold increase compared to the ABE reaction under identical conditions (Table 2, entry 1 versus entry 2). Additionally, the time course study for the IBE condensation reaction showed that the concentration of acetone was lower than that of isopropanol during the entire course of the reaction (see Supporting Information Figure 4 and Table 3). Note that the decrease in the rate of aldol reaction between in-situ-formed acetone and linear aldehydes led to a greater selectivity toward C12+ compounds over minor constituents of diesel fuel (C9–C11).

Given the favorable outcome in the chemocatalytic process of IBE, we explored the possibility of establishing a sustainable route to produce solvents for the IBE alkylation reaction from lignocellulosic sugars via Clostridial fermentation. It was recently reported that *C. acetobutylicum* would produce IBE by expression of the secondary alcohol dehydrogenase (SADH) from *Clostridium beijerinckii* strain B593.[18] Construction of a similar strain was achieved by combining an artificially synthesized SADH operon controlled under the acetoacetate decarboxylase promoter and terminator with a modified acetone or ACE operon containing the acetoacetate decarboxylase, acetyl-CoA, and butyryl-CoA transferase genes (*adc-ctfa-ctfb*, respectively) from *C. acetobutylicum*. The resulting strain (*sadh-adc-ctfa-ctfb*) or SACE produced isopropanol instead of acetone.[19] A 1.5 L batch fermentation of the SACE strain produced 21.5 g $L^{-1}$ of alcohols (6.2 g $L^{-1}$ isopropanol, 3.8 g $L^{-1}$ ethanol, and 11.5 g $L^{-1}$ butanol) from 54 g $L^{-1}$ of glucose in 35 h (see Supporting Information, Figure 5).

To integrate the biological IBE fermentation with the chemocatalytic approach, a selective, non-toxic, water-immiscible extractant was needed. Previous work showed glyceryl tributyrate to be an excellent extractant for ABE fermentation;[12] however, preliminary testing showed poor removal of isopropanol from an aqueous environment. Liquid–liquid equilibrium modeling with COSMO-RS (part of the COSMO*therm* software package) identified oleyl alcohol as a superior extractant for isopropanol and was confirmed by experiment (see Supporting Information, Table 5). Furthermore, oleyl alcohol was compared using COSMO-RS[20] with all previously reported ABE fermentation extractants[21–24] and was determined to be the best extractant on the basis of isopropanol removal with a $K_D = 0.8$. A 90-hour, 1.5 L fed-batch extractive fermentation of the SACE strain with a 1:2 volume ratio of medium and oleyl alcohol produced 39.6 g of alcohols, 5.6 g isopropanol, 17.9 g butanol,

**Table 2.** ABE versus IBE alkylation (See Scheme 1).

| Entry | Reaction | C5 [%] | 4-C7 [%] | 2-C7 [%] | C9 [%] | C10 [%] | C11 [%] | Alcohols[b] [%] | Yield [%] | C12+[c] [wt%] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ABE | 1.9 | 1.0 | 23.5 | 4.2 | 0.4 | 35.5 | 9.1 | 73 | 14 |
| 2 | IBE | 0 | 1.9 | 24.0 | 1.9 | 0 | 21.8 | 11.1 | 61 | 25 |

[a] Reaction conditions for ABE/IBE: acetone/isopropanol (2.3 mmol), butanol (1 mL), ethanol (1 mmol), 0.8 mol% Cu–HT, 240 °C, 14 h. Yield based on acetone determined by calibrated internal standard (*n*-dodecane). [b] Alcohols yield (%) for Entry 1: 2-C7-OH: 3.5; 4-C7-OH: 4.0; and C11-OH: 1.6. Entry 2: 2-C7-OH: 2.9; 4-C7-OH: 6.9; and C11-OH: 1.3. [c] Predominantly isomers of C13 and C15.
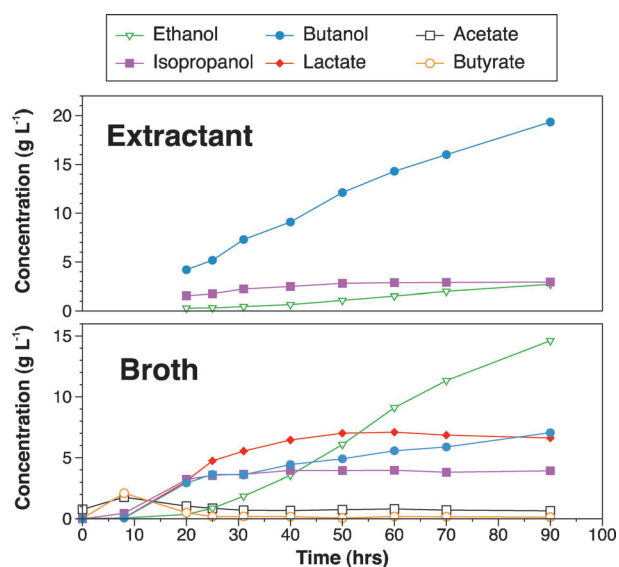
**Figure 3.** 1.5 L extractive fermentation with oleyl alcohol. Metabolites measured: acetate (black open square), butyrate (orange open circle), lactate (red diamond), isopropanol (purple square), 1-butanol (blue circle), and ethanol (green triangle).

and 16.1 g ethanol (Figure 3). These alcohols were produced from 117 g of glucose achieving an IBE yield of 0.34 g $g_{glucose}^{-1}$ or 78% of the theoretical maximum. Significant ethanol production was observed after isopropanol production ceased approximately 25 h into the fermentation. Alcohols distilled from the extractant phase of the fermentation were as reactive in the alkylation reaction as the previously tested synthetic mixture of IBE (see Supporting Information Table 4).[25]

In conclusion, we have leveraged advantages from both biological and thermochemical processes for the efficient conversion of sugar feedstocks to biodiesel. In the process, we have identified improved recyclable heterogeneous catalysts for the condensation of acetone and alcohols and demonstrated that an extractive IBE fermentation could be integrated with these catalysts. Thus, a significant increase in the production of desirable C12+ components was achieved by tailoring *both* the fermentation and the heterogeneous catalyst. While further tailoring of the fermentative product distribution toward higher butanol so as to align with chemical catalysis is desirable, these results demonstrate the benefits of a comprehensive approach to achieving synergy between chemical and biological processes.[26]

## Experimental Section

In a 12 mL Q-tube containing a stir bar, 3.2 wt% Cu–HT (0.8 mol%) or 2 wt% Pd–HT (1.7 mol%) was charged. To the reaction mixture, acetone or isopropanol (2.3 mmol), ethanol (1 mmol) and butanol (2 mL) were sequentially added. The Q-tube was sealed and the reaction mixture was stirred for 24 h at 240 °C in a pre-heated metal block. The reaction mixture was cooled to room temperature and dodecane (internal standard) was added. The reaction mixture was

diluted with tetrahydrofuran and the gas chromatography analysis of the reaction mixture was carried out.

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