LETTER

Integration of chemical catalysis with extractive fermentation to produce fuels

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Nearly one hundred years ago, the fermentative production of acetone by *Clostridium acetobutylicum* provided a crucial alternative source of this solvent for manufacture of the explosive cordite. Today there is a resurgence of interest in solventogenic *Clostridium* species to produce n-butanol and ethanol for use as renewable alternative transportation fuels[1-3]. Acetone, a product of acetone–n-butanol–ethanol (ABE) fermentation, harbours a nucleophilic α-carbon, which is amenable to C–C bond formation with the electrophilic alcohols produced in ABE fermentation. This functionality can be used to form higher-molecular-mass hydrocarbons similar to those found in current jet and diesel fuels. Here we describe the integration of biological and chemocatalytic routes to convert ABE fermentation products efficiently into ketones by a palladium-catalysed alkylation. Tuning of the reaction conditions permits the production of either petrol or jet and diesel precursors. Glyceryl tributyrate was used for the *in situ* selective extraction of both acetone and alcohols to enable the simple integration of ABE fermentation and chemical catalysis, while reducing the energy demand of the overall process. This process provides a means to selectively produce petrol, jet and diesel blend stocks from lignocellulosic and cane sugars at yields near their theoretical maxima.

Natural biological routes to produce alcohols (ethanol and n-butanol) from carbohydrates have been known for more than 100 years, and these compounds have been produced in fermentations at high titres ($\sim$100 and $15\,\mathrm{g\,l^{-1}}$, respectively) and at yields near their theoretical maxima. These low-molecular-mass compounds are primarily suitable as additives or in certain situations (for example, E100 flex fuel vehicles) as alternatives to petrol. Advances in metabolic engineering have enabled the biological production of several higher-molecular-mass jet and diesel fuel compounds from carbohydrates, but until now these processes have suffered from low titres and yields[4-6].

Here we propose a chemical route to convert fermentation products from a variety of renewable carbohydrate sources into hydrocarbons that can be used for petrol, jet fuel and diesel. Because solventogenic fermentation products have lower carbon numbers than are appropriate for these fuels, coupling chemistry can be used to produce molecules that are larger than these natural fermentation products, ideally achieved by exploiting the functionalities inherent in the starting materials[7-9]. The acetone, n-butanol and ethanol (ABE) mixture produced by *Clostridium acetobutylicum* in a 2.3:3.7:1 molar ratio (3:6:1 mass ratio) provides such a system. *C. acetobutylicum* is able to produce ABE from a variety of sugars and carboxylic acids[10], providing the flexibility needed to accommodate regionally specific feedstocks. The ABE products harbour both the nucleophilic α-carbons of the acetone and the electrophilic α-carbon of the alcohols. These paired functionalities enable us to construct higher alkanes from two-carbon, three-carbon and four-carbon precursors by the alkylation of acetone with the electrophilic alcohols. As shown in Fig. 1, alkylation under suitable conditions results in C$_5$–C$_{11}$ or longer-chain ketones, which may be

deoxygenated to paraffins. These paraffins, from pentane to undecane, are components of petrol, diesel and jet fuel.

Using a synthetic ABE mixture of pure acetone, n-butanol and ethanol, we investigated the double alkylation of acetone to obtain heptan-4-one (**B** in Fig. 1) (alkylation with ethanol), nonan-4-one (**D**) (alkylation with one molecule each of ethanol and butanol) and undecan-6-one (**F**) (alkylation with butanol) under transition-metal-catalysed conditions (Fig. 1)[11,12]. Two possible side reactions in the transition-metal-catalysed alkylation of acetone with primary alcohols are self-condensation of the alcohol through the corresponding aldehyde (Guerbet reaction)[13] and oligomerization of acetone into diacetone alcohol, mesityl oxide, cyclohexenones and other products[14]. In particular, self-condensation of acetone affects the overall efficiency of the desired process by lowering the ratio of ketone to alcohol in the reaction mixture. Similarly, if too much butanol reacts through the Guerbet pathway then not all of the acetone will be consumed; however, the Guerbet reaction does form higher branched alcohols that could themselves be valuable fuel precursors or be combined with acetone to form up to C$_{19}$ ketones (Supplementary Fig. 1). The transition metal and base catalysts should therefore be chosen to minimize these competing processes. As previously observed[15], we found that solid potassium phosphate tribasic (K$_3$PO$_4$) was the optimal base for the transition-metal-catalysed alkylation of acetone (Supplementary Table 1). Using solid K$_3$PO$_4$ as a heterogeneous base, we observed only minor amounts of products from acetone condensation (**E**) and no Guerbet products.

Our tests of different transition-metal catalysts (Ir, Ru, Rh, Pt, Pd and Ni) with K$_3$PO$_4$ in toluene for the alkylation reaction revealed that palladium was superior to the other metals (Supplementary Table 2)[15]. Further, trials of various palladium precursors showed a marked effect of the precursor on the outcome of the reaction (Supplementary Table 3). Although most of the palladium precursors examined catalysed the alkylation of acetone, Pd/C was significantly more active than the other palladium precursors at 110 °C in toluene.

Increasing the reaction temperature to 145 °C resulted in a minor improvement in the selectivity for doubly alkylated products, but the overall efficiency of the reaction declined with increasing temperature (Fig. 2a). Increasing the amount of base led to a marked improvement in both the selectivity for double alkylation and the overall yield. With 1.28 molar equivalents of K$_3$PO$_4$ with respect to total alcohols (ethanol and butanol) at 145 °C, an overall 86% molar yield, based on acetone as the limiting reagent, of identified ketone products was obtained (Fig. 2b). We envisaged that tuning the reaction conditions would afford selective monoalkylation of acetone to obtain petrol-range hydrocarbon chains. Consequently, alkylation was examined at various temperatures with only 0.32 molar equivalents of K$_3$PO$_4$. Under these conditions, pentan-2-one and heptan-2-one became the major products, with C$_5$–C$_7$ species accounting for nearly 50% of the overall yield, with respect to acetone as the limiting reagent. The best result
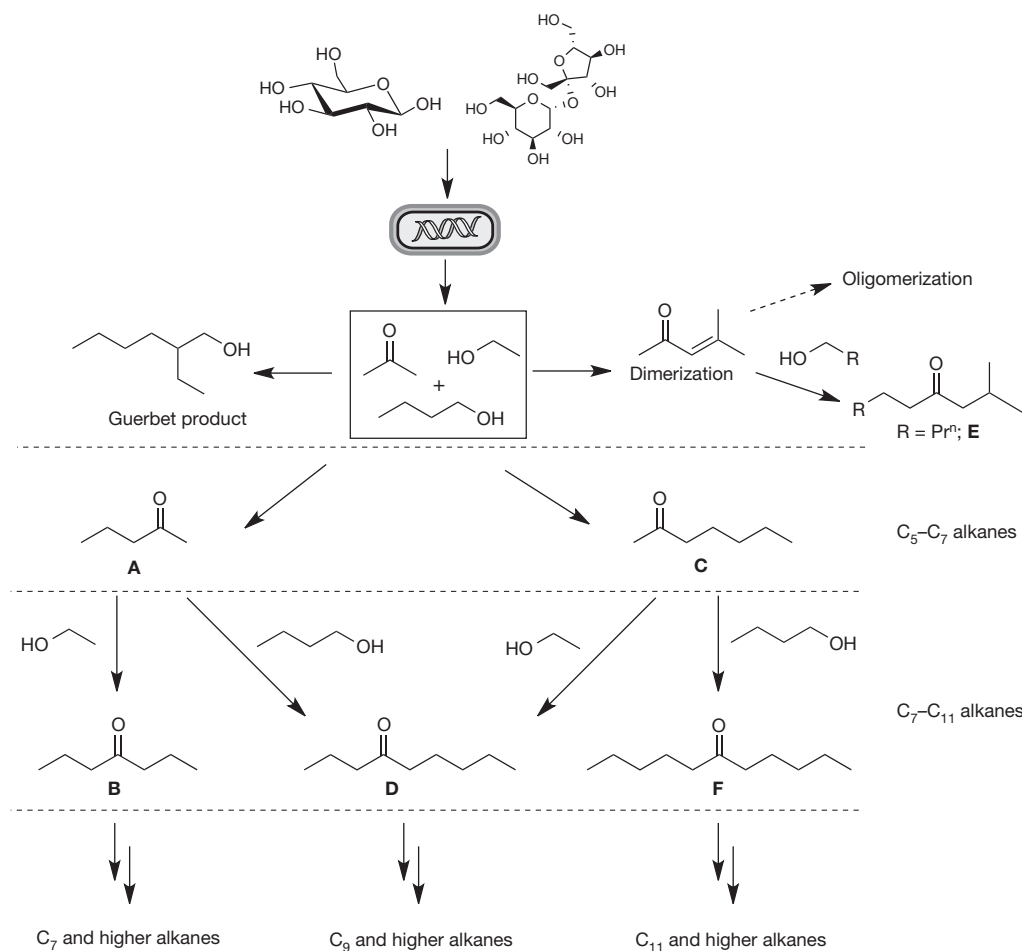
Figure 1 | A general approach to the transition-metal-catalysed production of biofuels from the ABE fermentation mixture.

was obtained at 145 °C with the production of pentan-2-one and heptan-2-one in 11% and 38% yield, respectively, along with 17% of double-alkylated products.

We explored the mechanism of the reaction by following the reaction progress. As shown in Fig. 2c, monoalkylation of acetone with butanol and ethanol occurred quickly within the first 1.0–1.5 h of the reaction to produce pentan-2-one and heptan-2-one. These species underwent further reaction to form double-alkylated products. No aldehydes were observed during the reaction, suggesting that the aldehyde intermediates were present in very low concentrations and reacted rapidly with acetone and other ketones. Hence, the formation of Guerbet products was minimized, and acetone alkylation predominated.

On the basis of our experiments and previous studies of the alkylation of N-nucleophiles and C-nucleophiles[11,16,17], we propose a mechanism for the double alkylation of acetone with ethanol and butanol (Fig. 3). The alcohols (butanol and ethanol) are dehydrogenated by the palladium catalyst, generating the reactive aldehydes and hydrogen. At this stage the aldehydes undergo either a self-aldol reaction to form the Guerbet product precursor, or an aldol reaction with acetone. Under the conditions employed here, condensation with acetone seems to be favoured, because acetone is present at much higher concentrations than the transient aldehydes. Subsequent dehydration of the aldol product under the reaction conditions furnishes α,β-unsaturated ketones that undergo palladium-catalysed hydrogenation with the hydrogen generated in the first step. Completing the same cycle with monoalkylated products affords the expected double alkylation products. The relative ease of attack by the unsubstituted versus the substituted α-carbon of the monoalkylated products leads primarily to

unbranched products. This combination of kinetic controls on the alkylation reaction therefore enables the conversion of a mixture of renewable metabolites into a well-defined range of ketone products.

One important aspect of any catalytic process is the lifetime of the catalyst, which we investigated by adding additional starting material after the reaction had been allowed to proceed to near completion. In a typical experiment using Pd/C and 0.96 molar equivalents of K_3PO_4 at 145 °C, we added an additional ABE mixture equivalent to the initial charge at 10 and 20 h after the beginning of the reaction and measured the overall yield at 10, 20 and 30 h. In this case, the overall yield based on the total acetone feed for each time point was only slightly decreased: 80% at 10 h, 72% at 20 h, and 61% at 30 h, demonstrating that the catalysts remained active and continued to convert each new amount of starting material. Furthermore, catalytic activity was maintained for 25 h under flow reaction conditions (Supplementary Fig. 4). The catalyst also retained activity in the absence of added solvent, achieving catalytic turnover numbers of more than 3,000 (moles of product per mole of palladium; Supplementary Tables 4 and 6). Although wet organic solvents could be used for the alkylation reaction, no reaction was observed in water. The addition of water was found to slow the overall reaction, resulting in lower double alkylation and overall yields (Supplementary Fig. 2).

To overcome the sensitivity of the catalysts to water, it was of interest to investigate highly selective water-immiscible extractants to remove acetone, n-butanol and ethanol *in situ*. *In situ* removal of the inhibitory product n-butanol during fermentation has been shown to increase solvent titres and yields, decrease distillation costs significantly, and decrease water use and reactor sizes[18–21]. However, the extractants typically employed are only capable of efficiently removing
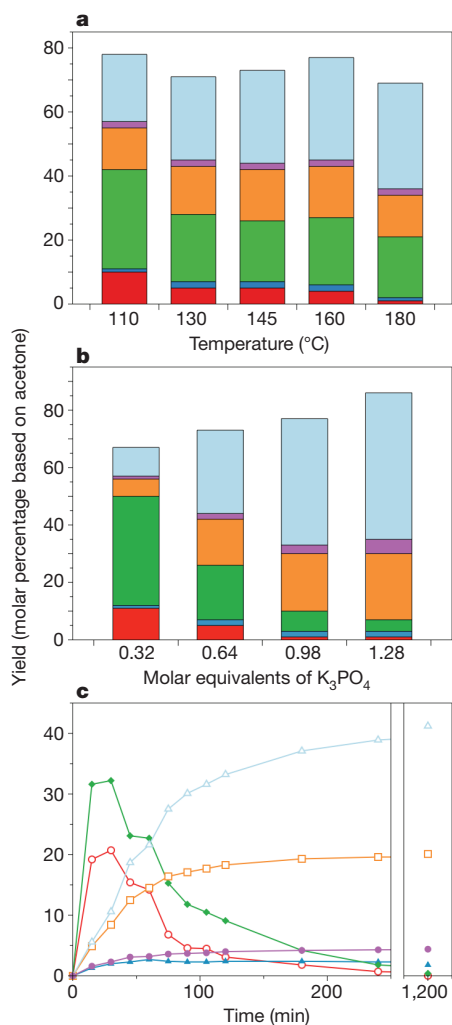
Figure 2 | Product distribution in Pd-catalysed alkylation as a function of reaction parameters and time course. a, Effects of temperature on product yields and distribution. Red, pentan-2-one; dark blue, heptan-4-one; green, heptan-2-one; orange, nonan-4-one; purple, 2-methylnonan-4-one; light blue, undecan-6-one. Yields based on acetone. Reaction conditions: acetone (2.3 mmol), ethanol (1 mmol), butanol (3.7 mmol), 5% Pd/C (0.01 mmol), K_3PO_4 (3 mmol), toluene (1.5 ml), temperature (*x* axis), 20 h. **b**, Effect of K_3PO_4 loading on product yields. K_3PO_4 loading is represented as molar equivalents with respect to total alcohol loading (ethanol + butanol). Reaction conditions were identical to the temperature studies with the exception of K_3PO_4 loading (*x* equivalents) and $T = 145\,°C$. **c**, Time course of product distributions during the palladium-catalysed alkylation reaction. Colours as in **b**. Reaction conditions: acetone (4.6 mmol), ethanol (2 mmol), butanol (7.4 mmol), 5% Pd/C (0.02 mmol), K_3PO_4 (9 mmol), toluene (3 ml), 145 °C.

acetone ($K_d = 1.1$) and n-butanol ($K_d = 2.6$) from aqueous solution[22]. Ethanol, however, remains preferentially in the aqueous phase ($K_d = 0.2$). In addition, glyceryl tributyrate efficiently removed several of the inhibitors of biofuel fermentation (for example furfural, *p*-coumaric acid and ferulic acid) found in acid-pretreated lignocellulosic biomass (Supplementary Table 5). This affords the simultaneous removal of residual inhibitors and the desired product during biofuel fermentation, a key advantage over other recovery technologies[23]. Studies of growth inhibition and cell viability with the use of up to 1:1 volume ratios of extractant to media showed glyceryl tributyrate to be non-toxic to *C. acetobutylicum* (data not shown). A 60-h 2-l fermentation of *C. acetobutylicum* on glucose with a 1:1 volume ratio of medium and glyceryl tributyrate produced 40.8 g of solvents with 16.4 g of n-butanol, 3.7 g of acetone and 0.8 g of ethanol, partitioning into the extractant phase (Fig. 4). These solvents were produced from 105 g of glucose and 1.6 g of acetate, achieving an overall ABE weight yield of 90% of the theoretical maximum. Similar results were observed with sucrose, the main carbohydrate of sugar cane, as the primary carbon source (Supplementary Fig. 5).

The *in situ* removal of ABE from the aqueous phase by a high-boiling-point extractant such as glyceryl tributyrate decreases energy requirements for product distillation[24] and allows integration of the biological and catalytic processes. Furthermore, any high-boiling impurities present in the extractant (for example furfural, *p*-coumaric acid or ferulic acid) would remain in the distilled extractant upstream of the catalyst, thus having no effect on catalyst performance. Solvents distilled from the extractant phase of the 2-l fermentation were as reactive as the pure chemicals, achieving an overall molar yield of

n-butanol from the aqueous phase, leaving most of the acetone produced in the aqueous phase. Glyceryl tributyrate was identified as a water-immiscible solvent capable of efficiently recovering both
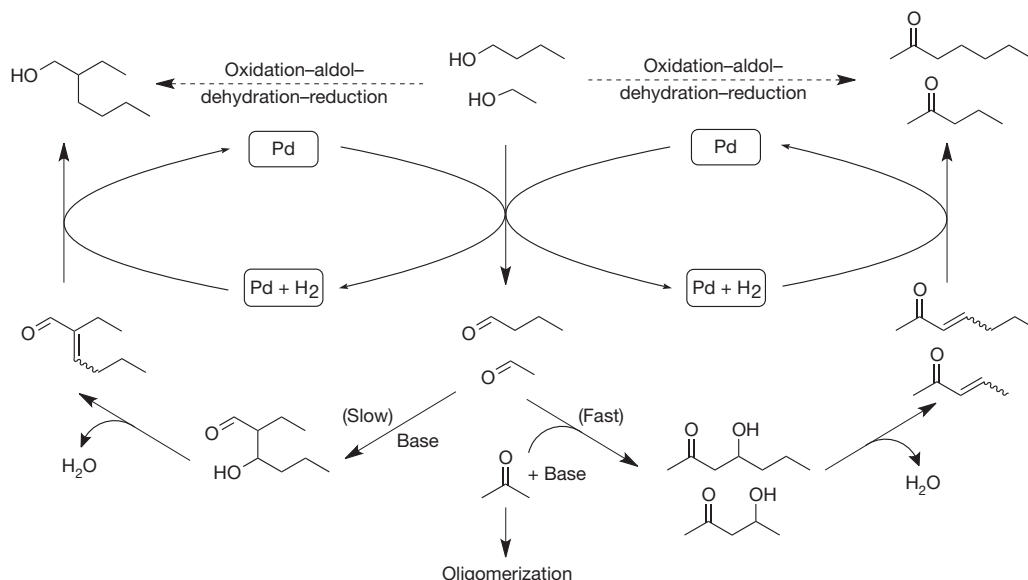

Figure 3 | Possible mechanism for the palladium-catalysed alkylation of acetone.

ABE fermentation | Solvent extraction | Distillation | Catalytic upgrade by dehydrogenation and aldol condensation
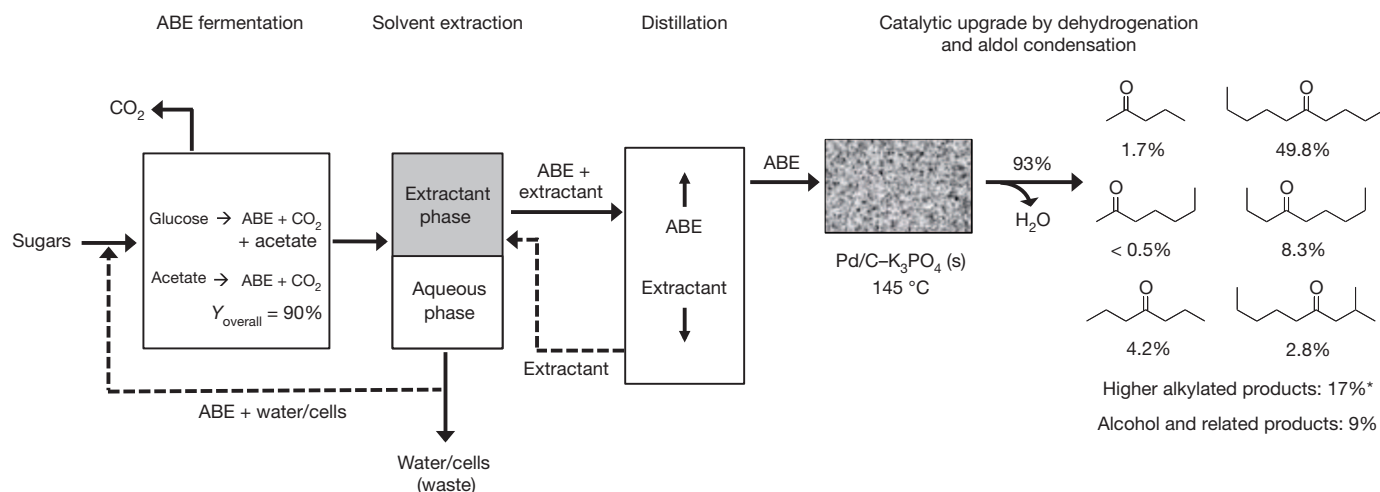

Figure 4 | Block flow diagram for integration of ABE fermentation with chemical catalysis. Dashed lines represent proposed recycle streams for continuous operation. The results correspond to a 2-l 60-h fed-batch extractive fermentation with glyceryl tributyrate to produce ABE from glucose. Alkylation reactions were performed on distilled solvents from the extractant phase after drying over molecular sieves; they were performed for 20 h in toluene at 145 °C with 1.28 molar equivalents of K_3PO_4. Yields are based on acetone. Asterisk, higher alkylated products and alcohol and related product yields were approximated by FID response factor and were assumed to have incorporated a single equivalent of acetone.

93% and 97% conversion of acetone (Fig. 4). Assuming complete recovery of ABE products from both the extractant and aqueous phases, ~20 g of C_7–C_{15} products would be formed, which represents ~38% of the carbon contained in the glucose feed (Supplementary Table 6). The combined selectivity of the fermentation and the alkylation results in this particularly high-yielding transformation of carbohydrates into fuel ketones. In comparison, a previous study[25] used similar aldol reactions to convert the alcohols and ketones in an aqueous-phase reformate into C_7 and higher species with a carbon yield of 42% from the intermediate oil feed and an overall carbon yield of 21% from the initial sorbitol feed. The lower yield of higher ketones resulted from the non-selective production of oxygenates that could not be upgraded in the aldol reaction. The 38% carbon yield in the present work does not account for the 15.1 g of lower-molecular-mass (C_4–C_6) petrol precursors also produced, which would then raise the overall carbon yield to ~58%. Further deoxygenation (Supplementary Fig. 6) of the products obtained from alkylation of the ABE mixture by using standard hydrotreating chemistry[25–27] would yield alkanes compatible with refinery infrastructure and suitable for blending with petrol, diesel and jet fuels.

Using this controlled alkylation of acetone, n-butanol and ethanol, we developed a high-yield method for transforming readily accessible fermentation products from a variety of carbohydrates into precursors for petrol, diesel and jet fuels. By catalytically upgrading low-carbon-number fermentation products we are able to exploit highly efficient metabolic pathways and achieve near theoretical yields[28]. Combined with the near theoretical yields attained during the alkylation reaction these higher-molecular-mass fuel precursors can be produced at relatively high titre. The tunability of this reaction to produce predominantly petrol or jet and diesel blend stocks is a significant advantage over other methods, and aligns well with current refining processes. Although further improvements will be required for commercial implementation, the results demonstrate that *in situ* extraction of the products from the ABE fermentation coupled with catalytic conversion of these products can provide hydrocarbon fuel blend stocks at high yields from biomass. The integration of extractive fermentation with chemical catalysis is thus a novel and potentially enabling route for the economical conversion of biomass into liquid transportation fuels.

METHODS SUMMARY

Transition-metal catalysis. All reactions were performed in closed systems using 12-ml Q-Tubes (pressure tubes) in a parallel optimizer and were analysed by gas chromatography–flame ionization detection, with dodecane as an internal standard. Reactions conditions (including temperature, catalyst and base loading) for individual alkylation reactions are described in Supplementary Methods.

Inhibitor extraction. Simulated clostridia fermentation medium as described in Supplementary Methods was used to calculate glyceryl tributyrate distribution coefficients. Acid-pretreated lignocellulosic inhibitors were produced from *Miscanthus × giganteus* as described in Supplementary Methods. The liquid hydrolysate was pH adjusted and inhibitor concentrations were analysed by gas chromatography–mass spectrometry.

Fermentation conditions. *Clostridium acetobutylicum* ATCC824 was routinely grown in clostridial growth medium (CGM) as described previously[1]. Fed-batch fermentations were conducted in 3-l bioreactors (Bioengineering AG) with a 2-l working volume. Additional glucose and yeast extract were added intermittently to the culture using a concentrated solution of 450 and 50 g l^{-1}, respectively. Cultures were grown anaerobically at 37 °C under pH control as described in Supplementary Methods. Sugars and major metabolites were measured in the aqueous phase by high-pressure liquid chromatography as described previously (Supplementary Fig. 5). Acetone, n-butanol and ethanol concentrations in the extractant phase were measured by gas chromatography–flame ionization detection (Supplementary Fig. 5).

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Supplementary Information is available in the online version of the paper.

Author Contributions Z.C.B. and P.A. contributed equally to this work. P.A. and S.S. performed experiments on the chemical catalysts, and E.G. performed experiments in the flow reactor. Z.C.B. optimized the fermentation and extractive processes. All authors contributed to the conception of the experiments, discussion of the results and preparation of manuscript.

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