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Combination of Bio- and Organometallic Catalysis for the Synthesis of Dioxolanes in Organic Solvents

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In the catalytic conversion of renewable raw materials, it is essential to adapt the reaction media to match the complexity of substrates. Recently, integrated bio- and organometallic catalysis processes have emerged; however, only a few operate in purely organic solvents, which would be advantageous for more energy-efficient processes. In this study, we present a process using one enzymatic step and two organometallic steps to produce the cyclic acetals 4,5-dipropyl-1,3-dioxolane and 4,5-dibutyl-1,3-dioxolane in a single organic solvent. The enzymatic step, in which a lyase is used, starts from the aldehyde, butanal orpentanal, and forms the 2-hydroxy ketones butyroin or valeroin, respectively. Subsequently, two organometallic steps were carried out sequentially in one reaction vessel. In a first step, the 2-hydroxy ketones are hydrogenated to 4,5-octanediol and 5,6-decanediol and in the second step the dioxolanes are formed by using hydrogen and carbon dioxide. Either formic acid or polyoxymethylene was used as an alternative carbon source to CO_2, which allowed considerable raw material flexibility. Since these dioxolanes are being investigated as additives in biofuel blends, the derived cetane number of the synthesized compounds was measured in addition to the viscosity and density. The cetane numbers determined suggest that the produced dioxolanes could be used as additives in fuel blends.

1. Introduction

Major efforts have been made to shift the dependence from the fossil fuels to more sustainable renewable alternatives.[1,2] An example of these efforts can be found in the USA and Brazil, where bioethanol as liquid fuel is produced starting from renewable resources such as corn or sugar cane in large scale.[3] In recent studies, cyclic acetals, especially dioxolanes, have been established as bio-hybrid fuel additives, that can flexibly combine the use of the renewable raw materials biomass, hydrogen (H_2), and carbon dioxide (CO_2).[4] An established synthesis route to structurally simple dioxolanes is presented *via* the reaction of formaldehyde and ethylene glycol.[5] Another new possibility for the synthesis of more complex dioxolanes starts from various aldehydes using a combination of bio- and organometallic catalysis.[6] The required aldehydes can be produced sustainably from bio-based sources, either through yeast-mediated production starting with glucose[7] or *via* enzymatic oxidation of bio-based alkanes to aldehydes using monooxygenases.[8,9] Specifically, it has been demonstrated that ten distinct *Cyanobacteria* strains are capable of producing alkanes.[8] Biocatalysis already plays an important role in organic synthesis and, in addition to its application in the pharmaceutical industry, is also increasingly used in the production of important basic chemicals.[10,11] One of the main advantages of biocatalysts is often their excellent selectivity, which is rarely achieved with other catalytic processes,[12] allowing the production of various pharmaceutically relevant chiral drugs.[13] Furthermore, enzymes are an effective tool for carrying out sustainable syntheses as they operate under mild conditions, achieve high yields and efficiencies, and are nontoxic, biodegradable catalysts.[11–16]

The combination of biocatalysis and organometallic catalysis was reported in many studies, for example, for lignin valorization,[17] or in vitro tandem catalytic reaction.[18] The integration of biocatalysis with organometallic catalysis incorporates the superior selectivity of biocatalysis with the high reactivity

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of organometallic catalysis, offering numerous advantages.[19] Nevertheless, also many challenges have been faced when combining these types of catalysis.[20] One of the most common challenges is to find a suitable reaction environment for both, enzymes and organometallic catalysis. On the one hand, most enzymes require the presence of water molecules for their activity. Hence, most enzymatic reactions take place in aqueous buffers.[21] On the other hand, most organometallic catalysts operate under organic conditions to avoid the catalysts inhibition or deactivation.[22] To overcome the compatibility problem, previous studies have taken different approaches by performing the reaction using bio- and organometallic catalysis in a biphasic system.[23,24] However, being able to perform reactions with both types of catalysis in the same solvent would simplify the process. Several approaches combining both catalysts in water have been described in the literature,[19,25] but these studies indicate that these systems do not represent the optimal solution for hydrophobic substrates. Therefore, a switch from aqueous buffer to organic solvent is recommended in some cases. Applying enzymes in organic solvents has already been demonstrated in the so-called micro-aqueous reaction system (MARS).[6,26,27] MARS allows the application of high concentrations of hydrophobic substrates and facilitates further downstream processing.[21] The system consists mainly of an organic solvent and the enzymes, which are formulated as lyophilized whole cells (LWC), forming a shell to protect the enzymes from the surrounding organic environment without losing activity or selectivity. A previous study demonstrated that the cells lose approximately 30% of their size during the water removal process. However, around 90% of the cells maintain their shape, although it is likely that the membrane becomes significantly poured.[28] Only a small amount of aqueous buffer is added, which is completely absorbed by the enzymes for their activation.[21] Cyclopentyl methyl ether (CPME) was used as a green solvent, as it was recently shown that it is suitable solvent for biocatalysis in MARS. CPME can be derived from the bio-based cyclopentanol or cyclopentanone and has several desirable properties including low peroxide formation rate, and a rather high boiling point of 106 °C.[29]

In this study, a combination of bio- and organometallic catalysis is used for the production of 4,5-dipropyl-1,3-dioxolane and 4,5-dibutyl-1,3-dioxolane starting from the bio-based aldehydes, butanal, or pentanal, respectively. Specifically, butanal can be synthesized directly from glucose by fermentation using *Clostridium acetobutylicum*.[30] Moreover, it has been shown that pentanal can be gained from apple fruit *via* 1-pentanol under anoxic and aerobic conditions.[31] In a previous study, the production of 4,5-dipropyl-1,3-dioxolane starting from butanal was presented using a two-step enzymatic process followed by a step catalyzed by an organometallic complex.[6] The introduction of both hydroxy groups *via* a lyase and an oxidoreductase allowed access to diols with high stereoselectivity, which was maintained during dioxolane formation. However, an evaluation of possible process routes showed that replacing the second enzymatic step with a step catalyzed by the same organometallic complex used for the third step can reduce the specific energy demand and maintain product concentrations.[32] In conclusion, when the aim is not the achievement of high selectivity but rather on economic effectiveness, the second step can be changed by an organometallic catalyst. Therefore, starting from butanal or pentanal, a ligation step was performed using the benzaldehyde lyase from *Pseudomonas fluorescens* (*Pf*BAL) to produce the respective 2-hydroxy ketone, namely butyroin or valeroin. After removing the CPME by distillation, the corresponding 2-hydroxy ketone was treated in a hydrogenation step catalyzed by the organometallic catalytic system [Ru(triphos)(tmm)]/$HNTf_2$ to form the corresponding diol. In a final step, the formed diol is used to synthesize the desired 1,3-dioxolanes. This step requires a C_1 building block which can

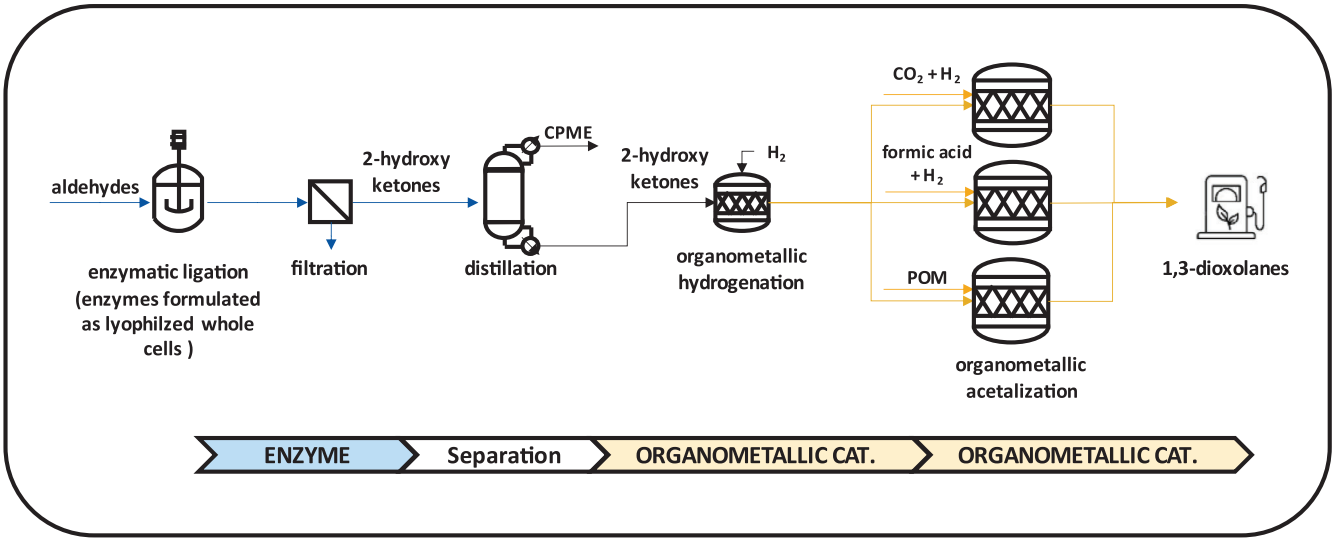


Figure 1. The route to 1,3-dioxolanes. Aldehydes are used as substrate for the first biocatalytic step to form a 2-hydroxy ketone. The latter is isolated by distillation. The resulting 2-hydroxy ketone is hydrogenated to the corresponding diol using the organometallic catalyst [Ru(triphos)(tmm)]/$HNTf_2$. In a final step, using the same organometallic catalyst, the diols are treated in an acetalization step by adding either CO_2 and H_2, or formic acid and H_2, or POM to obtain the desired 1,3-dioxolane.

be introduced *via* several routes. On a small scale the catalytic system comprised of [Ru(triphos)(tmm)]/$HNTf_2$ can be used to introduce either CO_2 or formic acid in combination with molecular hydrogen as a source for the carbon atom. At larger scales a Lewis acid catalyst allows for the use of polyoxymethylene homopolymer (POM-H) as C_1 source (Figure 1).

In this study, we show how the concentration of butyroin and valeroin can be increased by optimizing the reaction parameters. In addition, we were able to improve the overall conversion of 2-hydroxy ketones as an intermediate from aldehydes.

Furthermore, we demonstrate that either CO_2 or formic acid in combination with H_2, or polyoxymethylene (POM-H) can be used as C_1 building block in the synthesis of 4,5-dibutyl-1,3-dioxolane in addition to the 4,5-dipropyl-1,3-dioxolane as has been shown previously by Spöring et al.[6] Finally, the derived cetane number (DCN) of the synthesized dioxolanes was determined. DCN is one of the fuel properties that has a high impact on the combustion process and engine performance, where it was used as an indicator to measure the ignition quality of diesel fuels.[33]

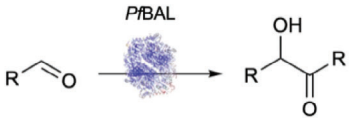
2. Results and Discussion

2.1. Process Optimization Toward High Titters of 2-Hydroxy Ketones in 1 mL

The 2-hydroxy ketones are obtained by forming a carbon–carbon bond from two aldehydes using *Pf*BAL as lyase (Scheme 1). Butanal and pentanal were used as substrates to form the desired 2-hydroxy ketones, butyroin and valeroin, respectively.

Initial concentrations of 53 mM and 52 mM were gained for butyroin and valeroin, respectively (Figures S1 and S2). Starting from these values, an optimization process was performed to enhance both, concentrations and conversions. The optimization process involved conducting the single enzymatic step by using *Pf*BAL while systematically altering one parameter.

The first parameter evaluated was the reaction system. Initially, a buffer system was used to accommodate the enzymes. However, due to the hydrophobic nature of the substrate, an organic solvent system using CPME was tested as an alternative. This change had enabled the application of a higher substrate concentration (400 mM instead of 200 mM) without having a second liquid phase and facilitated substrate feeding during the reaction process. Furthermore, the use of higher substrate concentrations necessitated extending the total reaction time to allow the enzymes sufficient time to interact with the substrate for highest conversion. As a result, different time points ranging from 1 to 4 days were evaluated. Additionally, two distinct mixing methods were compared to assess their impact on the overall interaction between the reaction components.



Scheme 1. Self-ligation of aliphatic aldehydes to 2-hydroxy ketones using *Pf*BAL as carboligase (R = propyl or butyl).

Table 1. Initial and optimized process parameters toward 2-hydroxy ketones.

Parameter	Initial	Optimized
Buffer/solvent	50 mM TEA	CPME
Substrate addition	Only initial substrate	Further feeding at different time points
Reaction time	1 day	4 days
New LWC	No	After 2 days
Substrate concentration	200 mM	400 mM
Mixing method	Shaking	Stirring

Hence, several parameters were tested individually as shown in Table 1. The optimization process was first carried out using only butanal as a substrate and afterward superimposed to the reaction with pentanal.

Through the optimization steps, an increase in both butyroin concentration and the total conversion was achieved (Figures 2 and 4). The greatest impact on the process improvement was achieved by changing the reaction medium from aqueous buffer to organic solvent (MARS). Butanal has a partition coefficient (log *P*) of 0.88.[34] The higher the log*P* the higher the hydrophobicity of the molecule. Hence, having a positive value of log*P* indicates a lower solubility in aqueous solution, resulting in a higher concentration in organic solvents.[35,36] Solving the solubility problem by using MARS allowed employing a higher starting substrate concentration. In addition, substrate feed was applied at different time points during the reaction.[6,21] Furthermore, extending the reaction time allowed the enzymes more time to consume the available butanal, increasing the total conversion of the process (Figures 2 and 4).

When the same optimized parameters were applied to the reaction starting from pentanal, an enhancement in product concentrations and conversions was observed (Figures 3 and 4). Pentanal was considered as a challenging substrate for two

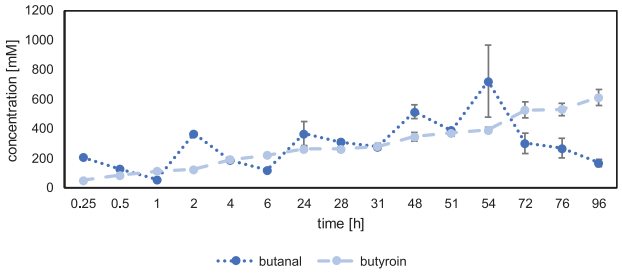


Figure 2. Optimized enzymatic step toward butyroin using *Pf*BAL in 1 mL; 400 mM butanal was used as initial substrate concentration. Additional 400 mM butanal was added after 2 h, 24 h, 48 h, and 54 h; 30 mg·mL^{-1} *Pf*BAL formulated as lyophilized whole cells were added in addition to 1 µL of 1 M TEA per 1 mg LWC to activate the enzymes. Another 30 mg·mL^{-1} *Pf*BAL were added after 48 h. The reaction was performed in MARS using CPME as the organic solvent for 96 h.

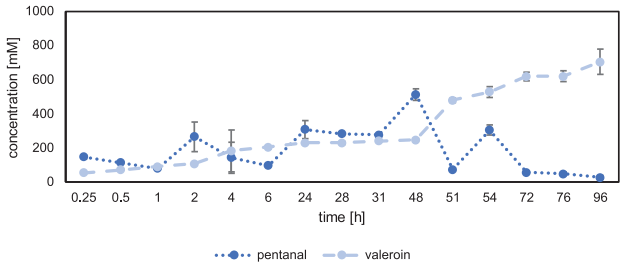


Figure 3. Optimized enzymatic step toward valeroin using *Pf*BAL in 1 mL; 400 mM pentanal was used as initial substrate concentration. Additional 400 mM pentanal was added after 2 h, 24 h, 48 h, and 54 h. The 30 mg·mL^{-1} *Pf*BAL formulated as lyophilized whole cells were added in addition to 1 µL of 1 M TEA per 1 mg LWC to activate the enzymes. Another 30 mg·mL^{-1} *Pf*BAL was added after 48 h. The reaction was performed in MARS using CPME as the organic solvent for 96 h.

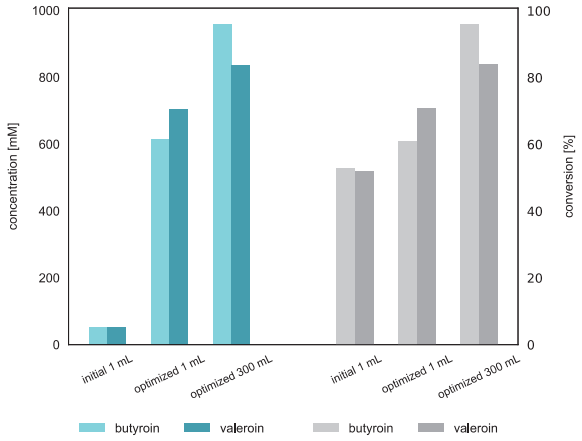


Figure 4. Comparison of concentrations and conversions for butyroin and valeroin between the initial and optimized conditions in 1 mL and 300 mL. The left Y-axis shows the concentrations of synthesized butyroin (in light blue) and valeroin (in dark blue) in [mM]. While the right Y-axis represent the conversions in [%] for butyroin (in light grey) and valeroin (in dark grey).

reasons. First, increasing the length of the carbon chain in aldehydes also increases hydrophobicity.[37] Second, having a longer carbon chain that makes it more difficult to reach the active site in the enzyme due to a steric hindrance. However, it was still possible to improve the concentration from 53 mM to 705 mM and the conversion from 53% to 71%. Changing the reaction medium also played an essential role, as the hydrophobicity of pentanal allows better dissolution in organic solvents. Since pentanal has a log *P* of 1.1, which is higher compared to butanal, an improvement was observed by replacing the aqueous buffer by organic solvent.

In both cases, no full conversion was achieved, although the mixturecontained almost no substrate at the end. This could be attributed to the volatile nature of pentanal, which may lead to a loss of some of the available pentanal through evaporation. Additionally, pentanal is a challenging substrate due to its long carbon chain, making it difficult to reach full conversion even after 24 h with the wild-type enzyme. Enzyme engineering, particularly at the active site, could potentially enhance the activity of the wild-type *Pf*BAL.

2.2. Process Optimization Toward 2-Hydroxy Ketones in 300 mL Scale

As mentioned above, 2-hydroxy ketones serve as precursors for dioxolanes in the production of bio-hybrid fuels. Therefore, it is necessary to be able to scale up the process to higher volumes. Hence, an enzymatic cascade toward butyroin and valeroin was carried out in an EasyMax device (Mettler Toledo) using the same optimized parameters as mentioned in Table 1. Almost full conversion of butanal to butyroin was achieved with a conversion of 96% (Figure 4 and Figure S3). In comparison, 835 mM of valeroin was produced with 84 % conversion (Figure 4 and Figure S4). Changing the mixing method from shaking (1 mL) to stirring (300 mL) using the EasyMax resulted in better distribution of the reaction component and thus a better interaction between the enzymes and the substrate. The results correspond to those by Brethauer et al. who showed that switching from shaking to stirring that gave better interaction with the substrate, leading to higher reaction rates.[38]

2.3. Scale-Up Approach Toward Valeroin in 400 mL Scale

For the determination of the DCN of the synthetic bio-hybrid fuels, derived from 4,5-dibutyl-1,3-dioxolane, an additional scale up was needed, as at least 50 mL of the corresponding dioxolane was required. Hence, a repeat of two 400 mL batches was performed (Figures S5 and S6).

During 92 h, pentanal was ligated to valeroin, reaching 904 mM and 828 mM in the 1st and 2nd batch, respectively. High conversions were also achieved with 90% and 83% for each batch, respectively.

Valeroin was isolated by using distillation. In this study, CPME was removed, and the product was concentrated to 68.15 mL (90%) and 62.84 mL (83%) for the 1st and 2nd batches, respectively (Table 2).

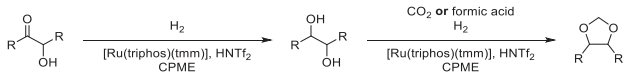
2.4. One-Pot Two-Step Process Toward Dioxolanes Using CO_2 or Formic Acid

Spöring et al. have already established the [Ru(triphos)(tmm)] catalyzed formation of dioxolanes from enzymatically produced vicinal diols starting from propanal or butanal with formic

Table 2. Theoretical, actual, and isolated volume of the synthesized valeroin out of the 1st batch and 2nd batch after product solation.

	1st Batch	2nd Batch
Theoretical volume	75.7 mL	75.7 mL
Actual volume	68.15 mL	62.84 mL
Isolated volume	90%	83%

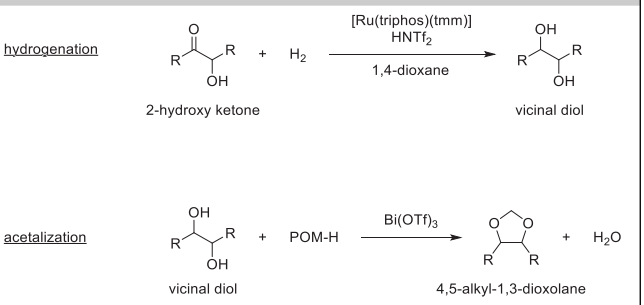
Table 3. One-pot two-step synthesis of dioxolanes starting from the enzymatically produced 2-hydroxy ketones.



Entry[a]	Substrate	Acetal Yield[b] (%) with CO_2	with Formic Acid
1	Butyroin	20.2	30.8
2	Valeroin	7.6	14.8

CO_2 or formic acid were used as C_1 source for the acetalization step.
[a] Reaction conditions: Hydrogenation: n([Ru]) = 0.005 mmol, $n(HNTf_2)$ = 0.005 mmol, n(substrate) = 0.7 mmol, V(CPME) = 2 ml, $p(H_2)$ = 100 bar. Acetalization: additional feed of n([Ru]) = 0.003 mmol, $n(HNTf_2)$ = 0.003 mmol, V(CPME) = 1 ml, n(formic acid) = 8 mmol or $p(CO_2)$ = 20 bar, $p(H_2)$ = 80 bar.
[b] Yields were determined by ^{1}H-NMR spectroscopy using mesitylene as internal standard.

Table 4. Larger scale synthesis of dioxolanes starting from the enzymatically produced 2-hydroxy ketones using POM-H as C_1 source for the acetalization step.



Entry[a]	Substrate	Isolated Acetalization Yield (%)
1	Butyroin	74
2	Valeroin	81

[a] Experimental details can be found in the Supporting Information.

acid or CO_2 as C_1 building block.[6] To demonstrate that the organometallic system can be utilized to replace the second reduction step in the enzymatic cascade, a one-pot two-step protocol for the synthesis of dioxolanes starting from the 2-hydroxy ketones was tested (Table 3).[39] The aim of the previous work was accessing high stereoselectivities by asymmetric synthesis with biocatalysis. In the present study, the focus was on the realization of a high product concentration since the main use of the obtained dioxolanes (4,5-dipropyl-1,3-dioxolane and 4,5-dibutyl-1,3-dioxolane) was in fuel applications. In the first step, the 2-hydroxy ketone is hydrogenated to the respective diol. After reloading with fresh catalyst and acidic co-catalyst, the C_1 building block was introduced into the reaction cascade in the second step.

Starting from butyroin (Table 3, entry 1) an acetal yield of 20.2% was obtained with CO_2 using this one-pot two-step process, while using formic acid as C_1 source resulted in a yield of 30.8%. These values are comparable to those obtained by Spöring et al. for the synthesis starting from 5,6-octanediol, with performing only one Ru-catalyzed step.[6] Interestingly, considerably lower acetal yields were obtained when valeroin was employed as substrate (entry 2). The reaction with CO_2 resulted in a yield of 7.6%, while 14.8% acetals were detected when formic acid was used. Higher yields were obtained with formic acid than with CO_2 as the latter needs to be hydrogenated to formic acid which then acts as the C_1 source to form the acetal.[38] The results demonstrate the general feasibility of using the 2-hydroxy ketones as substrates for the dioxolane synthesis with CO_2/formic acid. However, low acetal yields, especially when starting from valeroin, indicate significant room for optimization. In order to improve the yield of synthesized dioxolanes in future experiments, it is essential to shift the reaction equilibrium toward product formation. This can be achieved by increasing the availability of substrates, such as CO_2/H_2 or formic acid/H_2, either through higher initial concentrations at the start of the reaction or by continuous addition during the process. This approach is expected to enhance the amount of dioxolanes produced.

2.5. One-Pot Two-Step Process Toward Dioxolanes Using POM-H

Due to the low yield of acetal formation by using CO_2 or formic acid as C_1 source, another route was chosen to produce larger amounts of the cyclic acetals for fuel characterization. A two-stage reaction protocol was used, which also enables the use of plastic waste through the use of polyoxymethylene (POM) polymers (Table 4).

The first step of the synthesis was the hydrogenation of the ketone moiety using the catalytic system [Ru(triphos)(tmm)]/$HNTf_2$. The reaction was resulted in quantitative formation of the desired diols. The diols were then reacted to the respective acetals with POM-H as C_1 source under reaction conditions adapted from a paper published by Beydoun et al., using $Bi(OTf)_3$ as catalysts.[40] Vacuum distillation yielded the dioxolanes as colorless liquids. A good yield of 74% was obtained in the acetalization step when starting from 4,5-octanediol (starting from butyroin, Table 4, entry 1) and a very good yield of 81% with 5,6-decanediol (starting from valeroin, entry 2).

2.6. Determination of the Fuel Properties of Dioxolane

To provide an initial assessment of the fuel properties of the synthesized dioxolanes, the derived cetane number (DCN) was determined using an advanced fuel ignition delay analyzer (AFIDA) that measures the DCN based on the ignition delay time using a constant volume combustion chamber (CVCC, Table 5).[41,42]

Table 5. Derived cetane numbers for the synthesized acetals in comparison to conventional diesel fuel.

Entry	Fuel	DCN[a)]
1	Fossil diesel	>51[43]
2	4,5-Dipropyl-1,3-dioxolane	38.7
3	4,5-Dibutyl-1,3-dioxolane	56.2

[a)] DCN determined by AFIDA.

A DCN of 38.7 was measured for the butyroin-derived acetal (Table 5, entry 2), which is below the value of >51 required for fossil diesel in EN 590 (entry 1).[43] However, a DCN of 56.2 was determined for the valeroin-derived acetal, which meets the specification of fossil diesel fuel. The results demonstrate that the length of the two carbon side chains has a significant influence on the combustion properties of the acetals. This correlation allows for the fine-tuning of the combustion behavior toward a specific application, for example, as an additive to conventional diesel or for the use as pure fuel.

3. Conclusion

In recent years, many examples have been shown of the successful combination of biocatalysis and organometallic catalysis in various syntheses. However, many challenges had to be overcome, as each type of catalysis shows its highest activity in a different reaction environment. We have demonstrated a process using both types of catalysis (bio- and organometallic) in the green organic solvent CPME. CPME exhibits low toxicity to the enzymes when formulated as lyophilized whole cells and can be obtained from bio-based sources, making the presented synthesis route more environmentally benign.[6,21] Due to the high hydrophobicity of the substrates, namely butanal and pentanal, switching the enzymatic step from aqueous buffer to organic solvent allowed applying higher substrate concentrations up to 2 M. Similar enhancements were also demonstrated by Spöring et al. in the synthesis of diols starting from propanal or butanal, by switching to organic solvents.[6] As a result, an increase in the concentration of the 2-hydroxy ketones formed was achieved from 52 mM (butyroin) and 53 mM (valeroin) to 959 mM and 835 mM, respectively. In addition to that, the overall conversion was improved to 96% and 84% for butyroin and valeroin, respectively. Moreover, it was shown in a previous study, that using the same organic solvent instead of aqueous buffer in the enzymatic step made the transition to the organometallic step easier and less energy consuming.[32] Starting from the latter 2-hydroxy ketones, the corresponding diols and 1,3-dioxolanes were synthesized in a one-pot two-step process using the organometallic catalysis system [Ru(triphos)(tmm)]/$HNTf_2$. For the acetalization step, CO_2 or formic acid were used in combination with molecular hydrogen as C_1 source on a small scale. The use of formic acid as C_1 source showed higher yields for both dioxolanes as the hydrogenation step of CO_2 is not required. When POM was used as the C_1 building block on a larger scale, a significant improvement in yields was observed, reaching 74% and 81% for 4,5-dipropyl-1,3-dioxolane and 4,5-dibutyl-1,3-dioxolane, respectively. This high yield is important, as the latter dioxolanes are considered a good blend for bio-hybrid fuels. Sustainable resources can be integrated into the process when the acetal moiety is introduced using CO_2 from the atmosphere or CO_2-based formic acid with renewable hydrogen, or when waste POM-H is employed. Finally, the derived cetane number (DCN) of the fuels obtained from the synthesized 4,5-dipropyl-1,3-dioxolane and 4,5-dibutyl-1,3-dioxolane was investigated. The DCN resulted in values of 38.7 and 56.2, respectively. Since the minimum value of the current european standard for fossil diesel is 51, the latter dioxolane shows a high potential to play a role in the production of bio-hybrid fuels, making it appealing for further investigation and synthesis optimization.

4. Experimental Section

4.1. Biocatalyst Preparation

*Pf*BAL (GenBank AY007242.1) was expressed in *Escherichia coli* (*E. coli)* BL(DE3) (sequence in the Supporting Information) using an autoinduction medium within 5 L shaking flasks.[44] The cultivation conditions were set at 37 °C and 75 rpm for 2 h, after which the temperature was decreased to 20 °C and maintained for 48 h. Following cultivation, the cells were harvested by centrifuging at 7000 rpm and 10 °C for 40 min. The resulting cell pellets were stored at −20 °C before being lyophilized at −52 °C for 72 h under a pressure of 1.0 mbar. Post-lyophilization, the cells were finely grounded using a mortar and stored at −20 °C.

4.2. Setup of Enzymatic Carboligation Reaction Toward Butyroin and Valeroin in 1 mL Scale

In aqueous buffer: The carboligation process was conducted using a thermal shaker set to 30 °C and 1000 rpm. Within a 1.5 mL glass vial, 50 mM triethanolamine (TEA) pH 9 was combined with 15 $mg.mL^{-1}$ *Pf*BAL formulated as lyophilized whole cells as described above. To start the reaction, 200 mM pentanal was added. Samples were collected periodically at predetermined times.

In micro aqueous reaction system (MARS): The carboligation process was conducted using a thermal shaker set to 30 °C and 1000 rpm. Within a 1.5 mL glass vial, cyclopentyl methyl ether (CPME) was combined with 30 mg mL^{-1} *Pf*BAL formulated as lyophilized whole cells and 200/400 mM of pentanal. The reaction was initiated by adding 1 µL of 1 M TEA pH 9 per 1 mg LWC. Samples were collected periodically at the predetermined times. Additional 200/400 mM pentanal were fed at different time points.

4.3. Sample Preparation

For samples preparation, 20 µL of the reaction mixture was combined with acetonitrile and *n*-decane (380 µL, 0.16% v/v) serving as internal standard. The samples, placed in Eppendorf tubes, were centrifuged for 3 min at 14,000 rpm. Subsequently, 380 µL of the supernatant was taken for analysis using gas chromatography (GC).

Table 6. Retention time of used aldehydes and 2-hydroxy ketones in minutes.

Compound	Retention Time (min)	Compound	Retention Time (min)
Butanal	3.49	Pentanal	3.27
(*R*)-butyroin	9.435	(*R*)-valeroin	11.15
(*S*)-butyroin	9.53	(*S*)-valeroin	11.26

4.4. Scale-Up Approach in 300 mL and 400 mL

For scaling up to 400 mL volume, an EasyMax 402 system equipped with two 400 mL reactor vessels from Mettler Toledo was utilized. The same conditions were used as described for 1 mL scale in addition to the optimized paramteres in Table 1. The stirring speed was set at 250 rpm.

4.5. Instrumental Analytics and References

Samples were analyzed on a Thermo TRACE_1300_1310 GC with a CP-Chirasil-Dex CB (Agilent J&W) column (i.d. 0.25 mm; 25 m x 0.25 mm).

For the analysis of butyroin, a temperature gradient from 40 to 190 °C was used (40 °C hold 0.5 min, to 77 °C with 10 °C min^{-1}, hold 0.75 min, to 190 °C with 40 °C min, hold 5 min). The total run time was 13 min.

For valeroin, a temperature gradient from 77 to 190 °C was used (77 °C hold 1.25 min, to 190 °C with 10 °C min^{-1}, hold 3 min) with a total duration of 16 min.

For calibration, butanal, pentanal, racemic butyroin, and racemic valeroin were used. Retention times were as given in Table 6. The respective GC chromatogram of butyroin and valeroin are found in Figures S7 and S8 in the Supporting Information, respectively.

4.6. 2-Hydroxy Ketone Isolation

After scale-up, the LWCs were separated from the mixture by filtration using a vacuum pump. To remove the valeroin produced from the CPME, a custom distillation was prepared by using a Leybold DIVAC vacuum pump with a pressure of 10 mbar. The distillation of the valeroin was started at 120 °C and finished at 170 °C.

4.7. One-Pot Two-Step Synthesis of Cyclic Acetals from 2-Hydroxy Ketones Using a Ruthenium and Acid Catalyst[39]

The reactions were performed in 20 mL stainless-steel high-pressure autoclaves following a one-pot two-step protocol. The autoclave was degassed by evacuating and flushing with argon for at least three times. The catalysts [Ru(triphos)(tmm)] and $HNTf_2$ were weighed into a Schlenk tube in the glovebox and dissolved in CPME. Substrate was added and the mixture was stirred for 5 min and then transferred to the autoclave *via* a cannula in an argon counter stream. The autoclave was sealed, pressurized at room temperature with H_2 and heated to the reaction temperature using a preheated aluminum cone. After completion of the reaction time, the autoclave was cooled in an ice bath and carefully vented. Fresh catalyst and $HNTf_2$ were weighed into a Schlenk tube in the glovebox, CPME was then added, and the mixture was stirred for 5 min. If formic acid was used as C_1 source then, it was added to the fresh catalyst mixture. The fresh catalyst solution was transferred to the autoclave *via* a cannula in an argon counter flow. The autoclave was sealed and pressurized at room temperature with CO_2 (if applicable) and H_2 under stirring. The autoclave was then heated to the reaction temperature using a preheated aluminum cone. After completion of the reaction time, the autoclave was cooled in an ice bath and carefully vented. The reaction solution was analyzed by ^{1}H-NMR spectroscopy and the yield of the products was determined using mesitylene as an internal standard.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

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[1] A. T. Hoang, M. Tabatabaei, M. Aghbashlo, A. P. Carlucci, A. I. Ölçer, A. T. Le, A. Ghassemi, *Renewable Sustainable Energy Rev.* **2021**, *135*, 110204.
[2] R. Wolniak, B. Skotnicka-Zasadzień, *Energies* **2022**, *15*, 662.
[3] Y. Liu, P. Cruz-Morales, A. Zargar, M. S. Belcher, B. Pang, E. Englund, Q. Dan, K. Yin, J. D. Keasling, *Cell* **2021**, *184*, 1636–1647.
[4] M. Hellmuth, B. Chen, C. Bariki, L. Cai, F. Cameron, A. Wildenberg, C. Huang, S. Faller, Y. Ren, J. Beeckmann, K. Leonhard, K. A. Heufer, N. Hansen, H. Pitsch, *J. Phys. Chem. A.* **2023**, *127*, 286–299.
[5] A. Wildenberg, Y. Fenard, M. Carbonnier, A. Kéromnès, B. Lefort, Z. Serinyel, G. Dayma, L. Le Moyne, P. Dagaut, K. A. Heufer, *Proc. Combust. Inst.* **2021**, *38*, 543–553.
[6] J. D. Spöring, J. Wiesenthal, V. S. Pfennig, J. Gätgens, K. Beydoun, C. Bolm, J. Klankermayer, D. Rother, *ChemSusChem* **2022**, *16*, e20220198.
[7] H. G. Mengers, W. G. von Westarp, D. Brücker, A. Jupke, L. M. Blank, *Bioprocess Biosyst. Eng.* **2022**, *45*, 761–769.
[8] A. Schirmer, M. A. Rude, X. Li, E. Popova, S. B. del Cardayre, *Science* **2010**, *329*, 559–562.
[9] A. G. Katopodis, K. Wimalasena, J. Lee, S. W. May, *J. Am. Chem. Soc.* **1984**, *106*, 7928–7935.
[10] E. L. Bell, W. Finnigan, S. P. France, A. P. Green, M. A. Hayes, L. J. Hepworth, S. L. Lovelock, H. Niikura, S. Osuna, E. Romero, K. S. Ryan, N. J. Turner, S. L. Flitsch, *Nat. Rev. Methods Primers* **2021**, *1*, 46.
[11] S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem., Int. Ed.* **2021**, *60*, 88–119.
[12] A. R. Alcántara, P. Domínguez de María, J. A. Littlechild, M. Schürmann, R. A. Sheldon, R. Wohlgemuth, *ChemSusChem* **2022**, *15*, e202102709.

[13] G. Rossino, M. S. Robescu, E. Licastro, C. Tedesco, I. Martello, L. Maffei, G. Vincenti, T. Bavaro, S. Collina, *Chirality* **2022**, *34*, 1403–1418.
[14] J. B. Pyser, S. Chakrabarty, E. O. Romero, A. R. H. Narayan, *ACS Cent. Sci.* **2021**, *7*, 1105–1116.
[15] A. I. llanes, *Enzyme Biocatalysis: Principles and Applications*, Springer-Verlag, New York, NY **2008**.
[16] G. de Gonzalo, A. R. Alcántara, P. Domínguez de María, J. M. Sánchez-Montero, *Expert. Opin. Drug. Discov.* **2022**, *17*, 1159–1171.
[17] A. Z. Werner, L. D. Eltis, *Trends Biotechnol.* **2023**, *41*, 270–272.
[18] Y. Wang, H. Ren, H. Zhao, *Crit. Rev. Biochem. Mol. Biol.* **2018**, *53*, 115–129.
[19] H. Gröger, F. Gallou, B. H. Lipshutz, *Chem. Rev.* **2023**, *123*, 5262–5296.
[20] Y. Liu, P. Liu, S. Gao, Z. Wang, P. Luan, J. González-Sabín, Y. Jiang, *Chem. Eng. J.* **2021**, *420*, 127659.
[21] M. M. C. H. van Schie, J.-D. Spöring, M. Bocola, P. Domínguez de María, D. Rother, *Green Chem.* **2021**, *23*, 3191–3206.
[22] F. Rudroff, M. D. Mihovilovic, H. Gröger, R. Snajdrova, H. Iding, U. T. Bornscheuer, *Nat. Catal.* **2018**, *1*, 12–22.
[23] C. A. Denard, H. Huang, M. J. Bartlett, L. Lu, Y. Tan, H. Zhao, J. F. Hartwig, *Angew. Chem., Int. Ed.* **2014**, *53*, 465–469.
[24] M. Heidlindemann, G. Rulli, A. Berkessel, W. Hummel, H. Gröger, *ACS Catal.* **2014**, *4*, 1099–1103.
[25] H. Gröger, W. Hummel, *Curr. Opin. Chem. Biol.* **2014**, *19*, 171–179.
[26] A. Jakoblinnert, R. Mladenov, A. Paul, F. Sibilla, U. Schwaneberg, M. B. Ansorge-Schumacher, P. D. de María, *Chem. Commun.* **2011**, *47*, 12230.
[27] A. Jakoblinnert, D. Rother, *Green Chem.* **2014**, *16*, 3472–3482.
[28] J. Wachtmeister, A. Jakoblinnert, J. Kulig, H. Offermann, D. Rother, *ChemCatChem* **2014**, *6*, 1051–1058.
[29] G. de Gonzalo, A. R. Alcántara, P. Domínguez de María, *ChemSusChem* **2019**, *12*, 2083–2097.
[30] J. T. Ku, W. Simanjuntak, E. I. Lan, *Biotechnol. Biofuels* **2017**, *10*, 291.
[31] D. R. Rudell, D. S. Mattinson, J. P. Mattheis, S. G. Wyllie, J. K. Fellman, *J. Agric. Food Chem.* **2002**, *50*, 2627–2632.
[32] W. Graf von Westarp, J. Wiesenthal, J.-D. Spöring, H. G. Mengers, M. Kasterke, H.-J. Koß, L. M. Blank, D. Rother, J. Klankermayer, A. Jupke, *Commun. Chem.* **2023**, *6*, 253.
[33] K. Sivaramakrishnan, R. Paramasivam, *ARPN J. Eng. Appl. Sci.* **2012**, *7*, 205–2011.
[34] C. Hansch, A. Leo, D. H. Hoekman, *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*, *2*, ACS, Washington, DC **1995**.
[35] L. Xing, R. C. Glen, *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 796–805.
[36] A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **1971**, *71*, 525–616.
[37] X. Wang, T. Feng, C. Fan, X. Wang, S. Xia, J. Yu, C. J. Swing, *Food Chem.* **2023**, *426*, 136560.
[38] K. Beydoun, J. Klankermayer, *Chem. –Eur. J.* **2019**, *25*, 11412–11415.
[39] J. Wiesenthal, RWTH Aachen University **2023**.
[40] K. Beydoun, J. Klankermayer, *ChemSusChem* **2020**, *13*, 488–492.
[41] M. Neumann, J. G. Rittig, A. Ben Letaief, C. Honecker, P. Ackermann, A. Mitsos, M. Dahmen, S. Pischinger, *Energy Fuels* **2024**, *38*, 13264–13277.
[42] J. Luecke, B. T. Zigler, *Fuel* **2021**, *301*, 120969.
[43] M. Lapuerta, J. Rodríguez-Fernández, E. F. de Mora, *Energy Policy* **2009**, *37*, 4337–4344.
[44] Z. Li, W. Kessler, J. van den Heuvel, U. Rinas, *Appl. Microbiol. Biotechnol.* **2011**, *91*, 1203–1213.

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