

# Direct Electrosynthesis of 2-Butanone from Fermentation Supernatant

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## Abstract

Fossil-based products strongly attribute to the CO<sub>2</sub>-footprint of the chemical industry, which must decline to restrain the consequences of the anthropogenic climate change. This can be achieved by sustainable production processes from bio-based resources. We propose a sustainable production process for methy-ethyl-ketone (MEK), an abundantly used solvent. The process includes the biotechnological conversion of biomass to acetoin and the subsequent electrochemical reduction to MEK. Direct electrocatalytic reduction of acetoin in a fermentation supernatant without prior purification mitigates process complexity and increases process efficiency. We show that production of MEK from a fermentation broth is feasible with a slightly decreased yield compared to model solution experiments. Further, constant current experiments reveal a yield and selectivity dependence of the fermentation broth pH, which is not apparent in the model solution. Starting from 50 g L<sup>-1</sup> acetoin fermentation broth, we reach approximately 50 % yield of MEK with a product selectivity of 80 %. The conversion of acetoin is limited due to side product formation and hydrogen evolution reaction. This work contributes to the development of sustainable solvents to decrease CO<sub>2</sub>-emission in the chemical industry.

## Keywords

bio-process integration, green electrochemistry, methyl-ethyl-ketone (MEK), 2-butanone, electrochemical biomass upgrading, bio-hybrid fuel production, green solvent synthesis

# Introduction

Solvent production and utilization strongly contribute to the CO<sub>2</sub>-footprint of the chemical industry. It can be greatly reduced by replacing the basis of abundantly used solvents from the current fossil to a bio-based feedstock and implement a sustainable energy-efficient production pathway. The existing guidelines for a green chemistry offer a basis for the evaluation of these processes.<sup>1</sup>

MEK(methyl-ethyl-ketone) is a commonly used solvent with a market of over 1.3 million metric tons market size in 2015 and expected to grow up to 1.754 million tons by 2020.<sup>2,3</sup> It is an environmentally friendly solvent due to its low acute toxicity and eco-toxicity compared to other commonly used solvents.<sup>4,5</sup> Applications are in the paint industry or as a plastic welding agent.<sup>6,7</sup> Further, MEK has shown good properties as a possible energy carrier or bio-fuel.<sup>8</sup> Today, more than 92 % of MEK is produced from fossil resources via reduction of 2-butanol, mainly from butylene which originates for C-4 oil cuts.<sup>9,10</sup> The large scale production and application of this solvent comes with a strong lever to decrease CO<sub>2</sub>-emissions when replacing its feedstock and production route with a sustainable alternative.

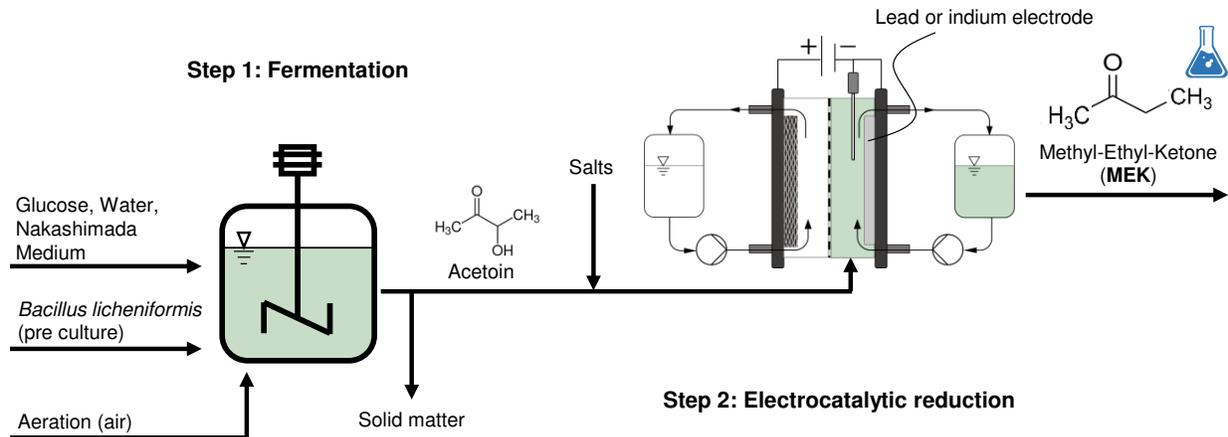
Several routes for the sustainable production of MEK from a biological feedstock are possible. Production based on microbial processes have been theoretically investigated<sup>11</sup> and experimentally conducted with, e.g., modified *E.Coli* with glucose<sup>12,13</sup> or levulinic acid as substrate,<sup>7</sup> and with *Klebsiella pneumoniae*<sup>14</sup> with the substrate glucose. However, yield and production rate remain low.<sup>15</sup> More promising results were achieved via 2,3-butanediol (2,3-BD) fermented from biomass with a subsequent chemical conversion.<sup>12,16–20</sup> Yet, the high boiling temperature and affinity to the aqueous fermentation broth of 2,3-BD leads to a high energy demand for the complex intermediate purification. Different approaches for an efficient purification of 2,3-BD from a fermentation medium, such as distillation, pervaporation, solvent extraction and reverse osmosis have been analyzed.<sup>21,22</sup> Lately, even more complex processes like anionic extraction based on the formation of reversible complexes,<sup>23</sup> extraction and subsequent purification with ionic-liquids<sup>24</sup> or liquid-liquid extraction coupled

with a thermal approach<sup>25</sup> were analyzed and further optimized to increase the efficiency.<sup>26</sup> Yet, the energy demand for these complex purification processes remains fairly high. The intermediate purification of these highly polar and low vapor pressure platform molecules like 2,3-BD or acetoin has been identified as one of the key challenges in biomass utilization.<sup>27</sup> A different route to produce MEK has been presented in 1984: In a two stage process, 2,3-BD was first electrochemically oxidized to acetoin, which was subsequently reduced to MEK.<sup>28</sup> However, this route was not pursued to technical maturity, because of the cost-efficient synthesis of MEK from fossil-based 2-butanol. In search for a sustainable production route, Ochoa-Gomez et al.<sup>29</sup> reintroduced the reductive electrochemical production route in 2019: Building on the advances in the microbial production of acetoin, they reduced acetoin to MEK using different electrode materials.<sup>29</sup> Promising figures of merit were achieved with an overall MEK yield of 60 % at an acetoin conversion of 72 % and a Faraday efficiency (FE) of 77 % for an initial concentration of 200 g L<sup>-1</sup> on a lead electrode was achieved with a selectivity of more than 80 % towards MEK. Unfortunately, the study did not move beyond model solutions and did not utilize fermentation broths.

As mentioned above, the fermentation of biomass to acetoin has been subject of detailed investigations. A variety of substrates like glucose<sup>30</sup> and galactose, mannitol and xylose as carbon sources,<sup>31</sup> but also less valuable compounds like hemicellulose/cellulose in a SSF (Simultaneous Saccharification and Fermentation) process have been identified.<sup>32</sup> From glucose, the highest reported titer known to the authors reached 100.1 g L<sup>-1</sup> acetoin,<sup>33</sup> with 2,3-BD as a substrate, 165.9 g L<sup>-1</sup> were reported.<sup>34</sup> Acetoin was also synthesized from mixed sugar substrates and oil palm fiber, or lignocellulosic hydrolysate,<sup>35-37</sup> lactose<sup>38</sup> and bakery waste with a titer of up to 65.9 g L<sup>-1</sup>.<sup>22</sup> A more detailed overview on different microorganisms forming acetoin can be found in literature.<sup>21,39,40</sup> These advances in substrate range, microbial productivity and scaleability of the fermentation process, paired with the promising results of Ochoa-Gomes et al.<sup>29</sup> utilizing model solutions demonstrate the high potential of a sustainable production route for MEK via acetoin.

Yet, for an efficient overall process via acetoin an efficient intermediate purification from the fermentation media is necessary. With the rather similar physico-chemical properties of acetoin and 2,3-BD (high miscibility with water, boiling point), the separation is similarly challenging.<sup>19,21</sup> The fermentation to acetoin also takes place in a complex fermentation medium, which includes trace metals, proteins, cellular matter and salts. Further, most of the fermentations yield not one, but a spectrum of intermediate and final products, often including acetoin and 2,3-BD. In other systems, the separation of intermediate products from a fermentation broth has also been identified as crucial, and different strategies were demonstrated. For carboxylic acids for example, a sequence with intermediate membrane purification has been presented.<sup>41</sup> Other approaches couple electrochemical reactions into the fermentation in so-called bioelectrochemical systems.<sup>42</sup> For the production of methylsuccinic acid from itaconic acid, Holzhäuser et al. showed a electrochemical reduction in the fermentation medium.<sup>43</sup> They reported a nearly unchanged selectivity and yield compared to a model solution in small scale batch experiments at a decreased FE, which they mainly attributed to reduction reactions with residual sugar.

In this work, we couple the biotechnological synthesis of acetoin to the subsequent electrochemical MEK production. We circumvent the complex separation task, by eradicating the necessity for most of the intermediate purification. Therefore, we employ the fermentation broth supernatant as the electrolyte after the addition of a salt as supporting electrolyte. The electrochemical conversion is conducted in a flow-cell at current densities of up to  $50 \text{ mA cm}^{-2}$  with different electrodes, acetoin concentrations and pH values. A schematic of the process is presented in Scheme 1. In order to identify challenges when switching from model solutions to fermentation broths, we conduct all experiments with both, a model solution and the fermentation broth with equal acetoin concentrations. Two electrocatalysts were selected for this study. Lead showed promising results in model solution experiments from Ochoa-Gomez et al.<sup>29</sup> Indium is introduced as an alternative, non-toxic electrocatalyst for the reaction. We analyze the electrocatalyst's activity for acetoin reduction in Linear Sweep



Scheme 1: Process scheme of the coupling of biotechnological and electrochemical conversion. Glucose is fermented to acetoin in step 1 and subsequently reduced electrochemically to MEK in step two. The fermentation broth is not elaborately purified. Only solids are removed and a salt is added as supporting electrolyte prior to the electrochemical conversion.

Voltammetry (LSV) experiments at different acetoin concentrations. Subsequently, quantitative MEK production is conducted in a flow cell with increasing acetoin concentration. At last, the pH is adjusted to increase the process efficiency, revealing different influences of the pH on the model solution and the fermentation broth.

## Experimental

### Materials

Indium and lead electrodes were purchased at Evochem and Alfa Aesar, respectively, with a purity of >99,99 %. Counter electrodes were titanium mesh electrodes, coated with iridium-oxide, purchased from Umicore Electroplating. All experiments were conducted with a PG-STAT302N from Metrohm Autolab. Fumapem<sup>®</sup> F-14100 cation exchange membranes were used for flow-cell experiments. Acetoin and potassium dihydrogenphosphate ( $K_2HPO_4$ ) were purchased from Sigma-Aldrich (purity >96 % and >98 % respectively), potassium hydrogen phosphate ( $KH_2PO_4$ ) and 1M  $H_2SO_4$  were purchased from Carl Roth (purity >99 %).

## Electrolyte Solutions

Experiments were conducted with a model solution (MS) and a fermentation broth supernatant (FB). The MS was prepared from DI water by adding salt ( $7 \text{ g L}^{-1} \text{ K}_2\text{HPO}_4$  and  $5.5 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$ ) and acetoin with the desired concentration. The acetoin concentration of the FB was adjusted by diluting the original FB with the  $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$  containing electrolyte solution. The electrolyte salt was added to the FB to ensure sufficient conductivity.  $1 \text{ M H}_2\text{SO}_4$  was added drop wise to adjust the pH value whenever necessary.

## Fermentation

Fermentation was carried out according to<sup>30</sup> in a Nakashimada medium<sup>44</sup> with a *Bacillus Licheniformis*. The substrate was glucose. To increase the acetoin yield, fermentation was prolonged to 110 hours. The resulting broth was centrifuged at 11000 rpm for 10 minutes to ensure a solid-free supernatant. HPLC analysis revealed a complex mixture of different products (acetoin, 2,3-butanediol, MEK), listed in Table S2 in the supporting information (SI) next to a chromatogram of the fermentation broth in Figure S1.

## Electrochemical Characterization

Linear sweep voltammetry (LSV) measurements were conducted in an undivided cell. Prior to each experiment, the electrodes were cleaned by subsequently rinsing with  $1 \text{ M HCl}$  and DI water. Measurements were performed at different acetoin concentrations for both, MS and FB. All LSV measurements were compensated for 80 % of the  $iR$ -drop, which was measured via EIS prior to each experiment. EIS was measured at the respective OCP from  $1 \text{ MHz}$  to  $1 \text{ Hz}$  with an amplitude of  $10 \text{ mV}$ .

LSV measurements were performed with a scanrate of  $0.05 \text{ V s}^{-1}$ . A HgE11 ( $\text{Hg}/\text{HgSO}_4$  with

sat.K<sub>2</sub>SO<sub>4</sub>) reference electrode from Meinsberg was used as reference electrode.

## HPLC-Analysis

Liquid samples were analyzed via Agilent 1200 HPLC, equipped with an organic acid resin column from cs-chromatographie at 70°C, with 0,5 ml/min flow rate of 2.5 mmol H<sub>2</sub>SO<sub>4</sub> in H<sub>2</sub>O at a pressure of 45 bar. Detection is done via RID for 2,3-BD, MEK and 2-butanol. Acetoin is analyzed with a VWD at 190 nm wavelength. The respective retention times are displayed in Table S2 in the SI.

## Flow Cell Experiments

Flow cell experiments were conducted with the self-made flex-e-cell ([www.flex-x-cell.com](http://www.flex-x-cell.com)). A planar electrolysis-cell with an active electrode area of 35 cm<sup>2</sup> and an electrolyte gap of 2 mm with spacer was used. Electrolytes were pumped by gear pumps (MCP-Process, Colepalmer) with a flow rate of 100 mL min<sup>-1</sup> and recycled in the electrolyte tank. Samples were taken from the anolyte and catholyte tank periodically and analyzed via HPLC. All experiments were conducted at a current density of 50 mA cm<sup>-2</sup>. The apparent voltage on the working electrode was measured with an mini HydroFlex (R) reversible hydrogen electrode (RHE) from Gaskatel.

Experiments were evaluated for conversion of acetoin, selectivity towards MEK, 2,3-BD and 2-butanol, as well as FE. Because the individual experiments were conducted with varying initial amounts of acetoin, all results are compared at the time of 100 % theoretical conversion of acetoin. We define 100 % theoretical conversion as the time  $t_{TC}$ , when sufficient charge has passed the electrode to convert all acetoin to MEK with the assumption of no side or parasitic reactions.  $t_{TC}$  is calculated via equation 1, where  $c$  is the initial concentration of acetoin,  $V$  the electrolyte volume,  $M_{Acetoin}$  the molar mass of acetoin,  $z$  the number of

transferred electrons (2), F the Faraday constant and I the applied current.

$$t_{TC} = \frac{(c \cdot V)}{M_{Acetoin}} \cdot \frac{zF}{I} \quad (1)$$

Reactants crossing the membrane during the experiments were included in the experiment's evaluation, so that e.g., MEK in the anolyte is included in selectivity and yield. Changes in electrolyte volume due to osmotic drag were measured and accounted for when evaluating concentrations in the electrolyte.

## Results and Discussion

### Catalyst Analysis

Two different catalysts were characterized for electrochemical MEK synthesis: lead has yielded promising results in a study by Ochoa-Gomez et al.<sup>29</sup> and indium was additionally chosen due to its high overpotential for the hydrogen evolution reaction. Figure 1 displays LSV measurements with varying acetoin concentration in the model solution (MS) and in the fermentation broth (FB).

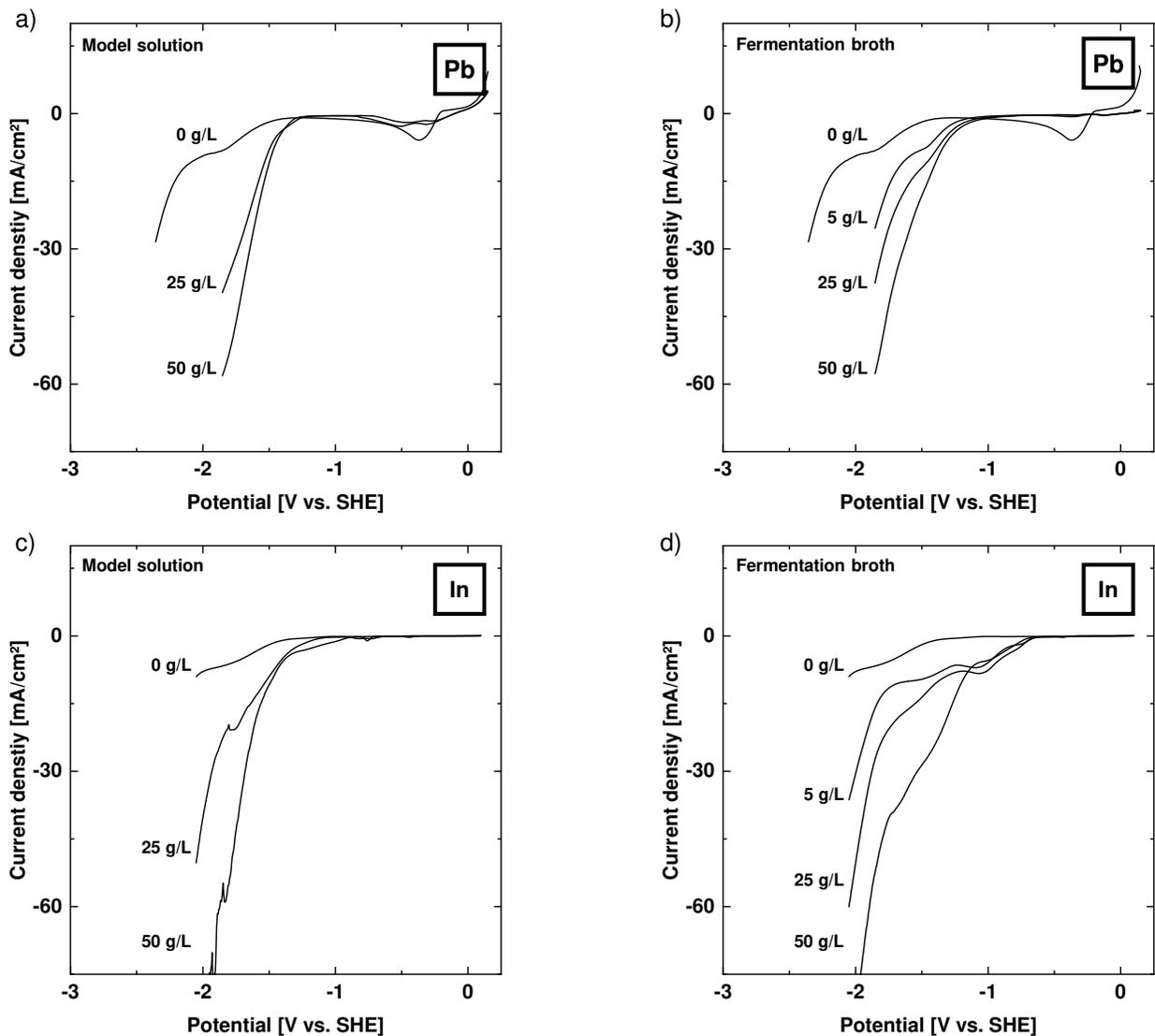


Figure 1: LSV on a lead electrode in a) model solution and b) fermentation broth and on an indium electrode in c) model solution and d) fermentation broth with increasing acetoin concentration at pH 3.2. The scan-rate was  $50 \text{ mV s}^{-1}$ .

As expected, the LSV measurements show a remarkable onset potential difference with and without acetoin in the solution. The onset potential decreases from about  $-1.5 \text{ V}$  vs SHE (Standard Hydrogen Electrode) for HER (Hydrogen Evolution Reaction) to  $-1.25 \text{ V}$  vs SHE when acetoin is present in the solution with a lead electrode in the MS in Figure 1 a). It decreases further with  $50 \text{ g L}^{-1}$  initial acetoin concentration. Additionally, the current density increases significantly with increasing acetoin bulk concentration after the onset potential

is surpassed. Similar results are visible for LSV-measurements with the indium electrode in Figure 1 c). The onset potential decreases from HER at  $-1.4\text{ V}$  vs SHE to  $-1.1\text{ V}$  vs SHE when acetoin is present in the solution, and decreases further with increasing acetoin concentration. The high overpotential of lead for HER opens a potential window for acetoin reduction.

LSV measurements were also conducted with the FB. The results are displayed in Figure 1 b) and d) for lead and indium, respectively. The general course of current is similar for measurements conducted with the MS. However, a notably lower onset potential is visible on both electrodes in the FB. At this onset potential, the initial increase in current is less steep compared to the MS and a plateau-like region is apparent. We attribute this behavior to side-reactions species in the FB, which are not present in the MS. Also, HER can be catalyzed by the various trace metals from the fermentation medium. The composition of the FB prior to the fermentation with the various trace components can be found in the Supporting Information in Table S1. Nevertheless, beyond this plateau-like region around the onset potential in the MS, a steep increase in current can be seen. The apparent current density is similar to the respective current density in the model solution, which indicates a similar reaction rate in the MS and the FB. We attribute this to a predominant reduction of acetoin on both electrode materials. Quantitative product analysis needs to be conducted in flow-cell experiments to give insight into product selectivity and to assess the catalysts suitability.

## Flow Cell Experiments

Quantitative analysis of the conversion, selectivity and FE was conducted in flow cell experiments. To assess the influence of an increasing acetoin concentration, experiments were initially conducted with diluted FB and MS. The degree of dilution was subsequently decreased, until the undiluted fermentation broth was employed. Figure 2 a) and b) depicts the concentrations of acetoin, MEK, 2-butanol, and 2,3-butanediol over time for MS and

FB with  $5 \text{ g L}^{-1}$  initial acetoin concentration on indium electrodes. The reaction pathways including the side products MEK, 2-butanol and 2,3-butanediol are displayed in Figure S2 in the Supporting Information. The qualitative trends are similar for other initial acetoin concentrations and the lead electrode.

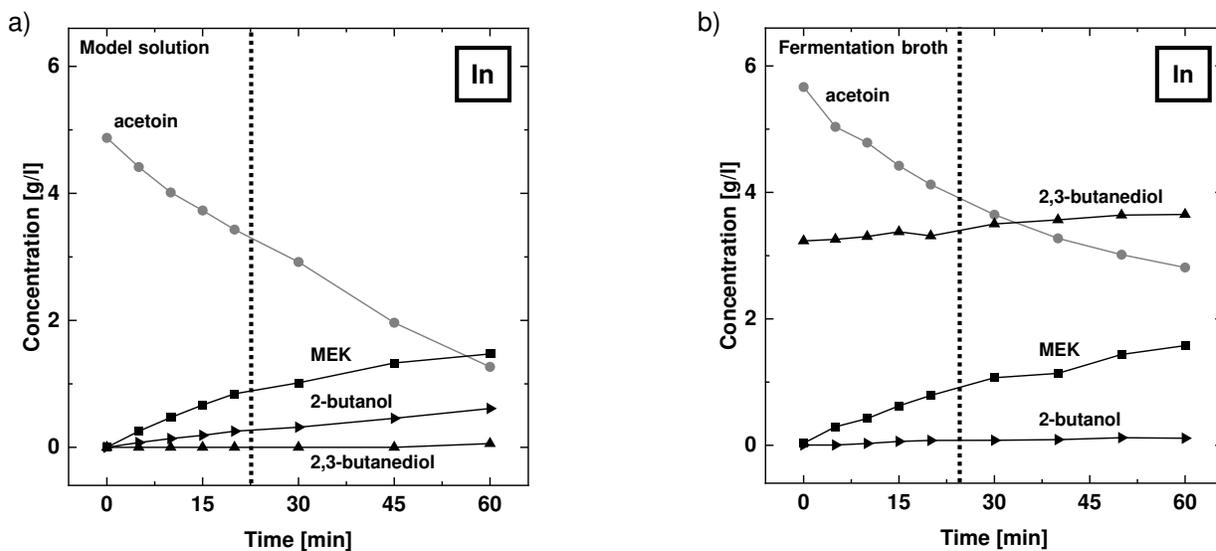


Figure 2: Concentration of reactants over the reaction time in a) model solution and b) fermentation broth at  $50 \text{ mA cm}^{-2}$  with an indium electrode. Dashed vertical line indicate ( $t_{TC}$ ).

In both experiments, the conversion of acetoin is apparent. In Figure 2 a), about 37 % of the initial acetoin is converted in the MS at 100 % theoretical conversion  $t_{TC}$ , as indicated by the dashed line. Bubble formation was observed on the cathode, indicating HER as parasitic reaction. Due to the low initial acetoin concentration, side reactions dominate and the FE is low (26 %). The reaction yields predominantly MEK with a selectivity (S) of 71 % and the side product 2-butanol. The 2,3-BD concentration remains close to zero over the entire time course.

In the diluted FB, the conversion of acetoin is 31 % at  $t_{TC}$  and lower as compared to the MS. The main product of the acetoin reduction is again MEK. Its selectivity (S) of 61 % is close to the selectivity in the MS experiment. 2,3-BD is present from the start of the experiment

as a byproduct of the fermentation. In contrast to the model solution, 2,3-BD is formed as the secondary product at a low rate. As in the MS experiment, bubble formation indicates HER as a parasitic reaction. The findings from these experiments at a low acetoin concentration match indications from LSV experiments, especially the more pronounced HER or side-reactions in the experiments with the fermentation broth.

### Influence of the Acetoin Concentration

Further experiments were conducted at higher acetoin concentration of up to  $50 \text{ g L}^{-1}$ . All experiments are benchmarked for conversion, selectivity, yield and FE after at the  $t_{TC}$ , so that the FE equals the yield. These parameters are displayed for indium in Figure 3 and for lead in Figure 4.

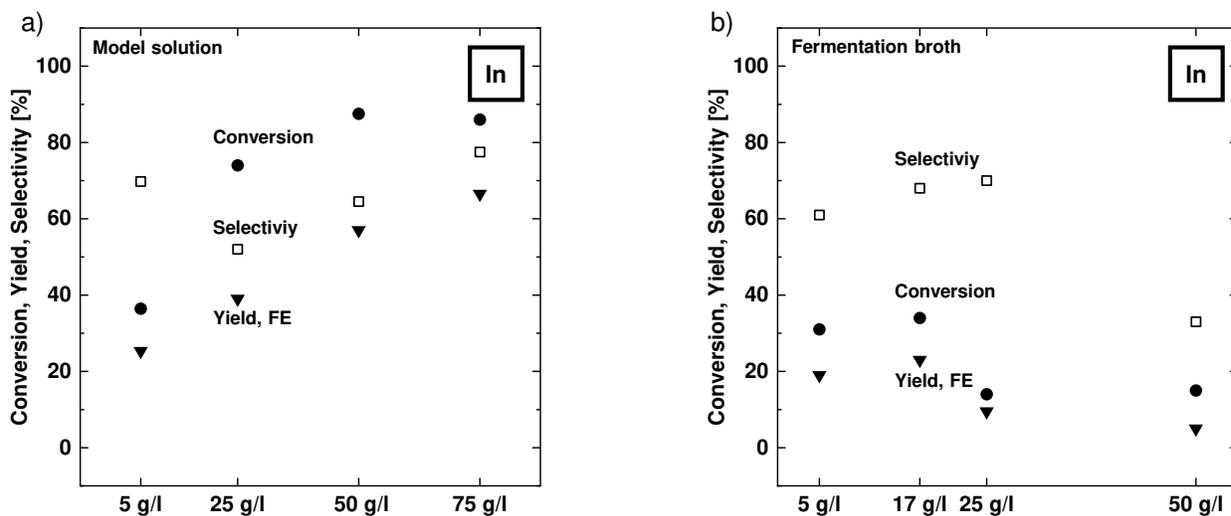


Figure 3: Conversion (●), selectivity (□) and yield (▼) of acetoin reduction to MEK at different initial acetoin concentrations in  $7 \text{ g L}^{-1} \text{ K}_2\text{HPO}_4$  and  $5.5 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$  at pH 3.2 in a) model solution and b) fermentation broth experiments on an indium electrode. Characteristic values are recorded at 100 % theoretical conversion ( $t_{TC}$ ).

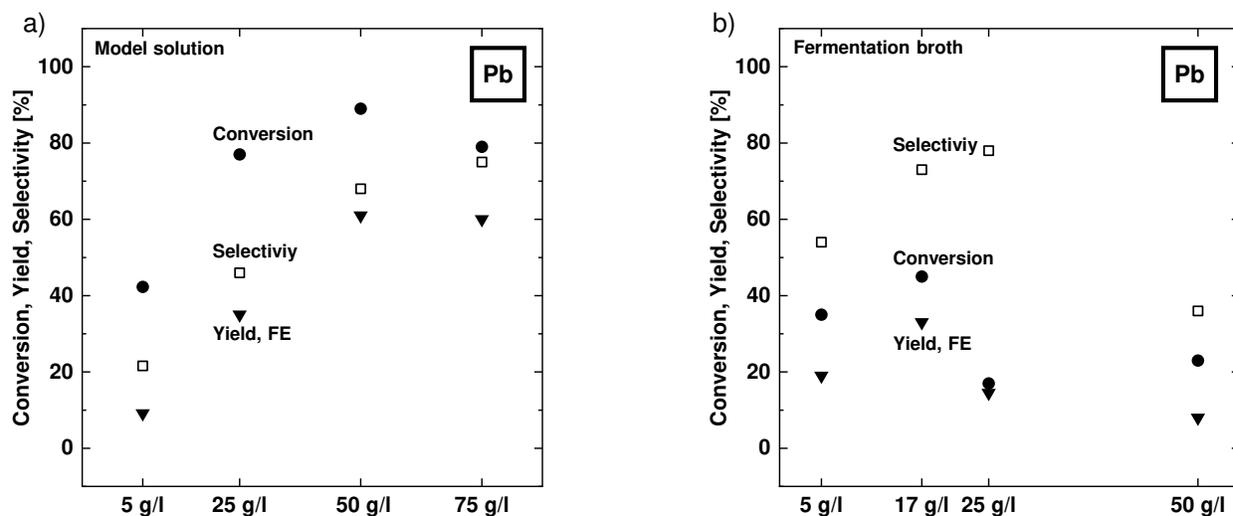


Figure 4: Conversion (●), Selectivity (□) and Yield (▼) of acetoin reduction to MEK at different initial acetoin concentrations in  $7 \text{ g L}^{-1} \text{ K}_2\text{HPO}_4$  and  $5.5 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$  at pH 3.2 in a) model solution and b) fermentation broth experiments on a lead electrode. Characteristic values are recorded at 100 % theoretical conversion ( $t_{TC}$ ).

Figure 3 a) displays the influence of an increasing acetoin concentration on selectivity, conversion and yield (FE) of MS experiments with an indium electrode. All indicators benefit from an increasing acetoin concentration in the MS. At an initial acetoin concentration of  $50 \text{ g L}^{-1}$ , experiments show a selectivity of  $S=64.5 \%$  and a yield and FE of  $Y=57.5 \%$  at a conversion of  $87.5 \%$ . A further increase in concentration to  $75 \text{ g L}^{-1}$  further increases selectivity to  $77.5 \%$  and yield to  $66.5 \%$ , while the conversion ratio of acetoin slightly decreases to  $86 \%$ .

The results of conversion from the gradually less diluted FB are depicted in Figure 3 b). The trend of increasing efficiency with an increasing reactant concentration does not extend to the FB. While selectivity, conversion and yield increase up to  $17 \text{ g L}^{-1}$ , they decrease for concentrations exceeding  $17 \text{ g L}^{-1}$ . At  $50 \text{ g L}^{-1}$  the yield  $Y$  is as poor as  $5 \%$ . Figure 4 a) and b) show the corresponding measurements with lead as the cathode and display a nearly identical trend.

On both electrodes, the acetoin concentration on the electrode's surface seems to be the

limiting factor for a concentration below  $50 \text{ g L}^{-1}$  at the given current density in the MS without impurities. Next to the process indicators, we also note a decreasing selectivity and FE over the course of the experiments, as acetoin is gradually depleted, depicted in Figure S3 in the supporting information. This fits findings from LSV-experiments, which show an increasing activity with increasing reactant concentration.

In the FB experiments, the low overall conversion rate of acetoin at a concentration above  $17 \text{ g L}^{-1}$  indicates more pronounced parasitic reactions, most likely a predominant HER. This is further supported by the increased bubble formation on the cathode which further increased formation of foam. Above  $25 \text{ g L}^{-1}$ , the selectivity decreases drastically, diminishing the overall process yield. The course of selectivity, conversion, yield and FE over the time of the experiments on a lead electrode at pH 3.2 is further depicted in S4. Next to the decreased product yield, the resulting foaming makes process control complex. Most likely, the increased HER in the FB can be attributed to the increased catalytic activity of various trace metal components in the mixture which can be seen in EDX-images of the electrode's surface after FB experiments in Figure S5 in the SI. Therefore, the reaction conditions chosen here seem to be unfavorable for conversion in an undiluted fermentation broth.

### **Influence of the pH Value**

Originally, the pH was chosen in accordance with Ochoa-Gomez et al.<sup>29</sup> from MS experiments. To suppress unwanted HER in the FB, we omitted the pH adjustment of the FB and continued experiments at a pH of 6.5. This decreases the intermediate treatment steps further. To show the influence of the pH on the MS, experiments with the MS were conducted accordingly.

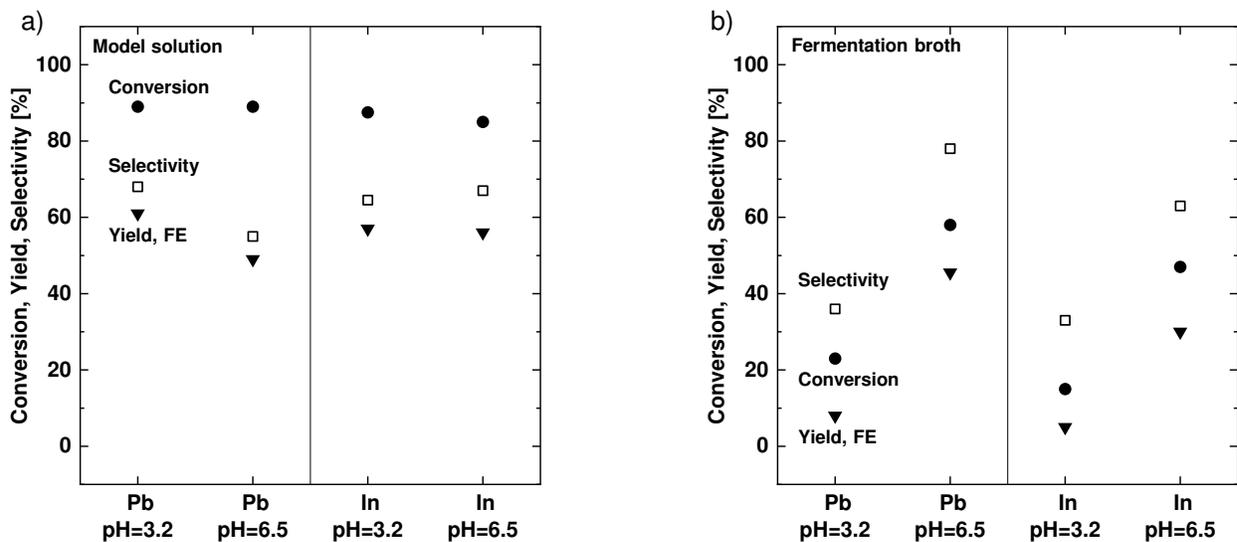


Figure 5: Conversion (●), selectivity (□) and yield (▼) of acetoin reduction to MEK at different pH in  $7 \text{ g L}^{-1} \text{ K}_2\text{HPO}_4$  and  $5.5 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$  in a) model solution and b) fermentation broth experiments on a lead electrode at  $50 \text{ g L}^{-1}$  initial acetoin concentration. Characteristic values are recorded at 100 % theoretical conversion ( $t_{TC}$ ).

Ochoa-Gomez et al.<sup>29</sup> describe the pH as a minor factor for the characteristic performance indicators and find the best performance at pH values between 3 and 5.5. Below a pH of 3 HER increases significantly<sup>29</sup> and above a pH of 7, aldol condensation is reported in literature.<sup>29,45</sup> Results for the MS in Figure 5 a) show, that reaction at a pH of 6.5 does not impair yield or selectivity on the indium electrode, while the selectivity on the lead electrode is slightly lower than at a pH of 3.2.

The influence of the pH on the reaction in the FB is displayed in Figure 5 b). In contrast to the MS, the pH has a major impact on the performance in the FB. All characteristic values, especially on the lead electrode, increase dramatically at this elevated pH, resulting in a yield and FE of  $Y,FE=47 \%$ . For indium, the increase in performance is less pronounced but still notable, with a yield and FE of  $Y,FE=30 \%$ . Further, bubble formation decreased dramatically which simplifies the process control significantly by decreased foaming.

As discussed before, HER was far more pronounced in the FB than in the MS at the pH of 3.2. We attribute this to active sites that might stem from deposited trace metals in the FB

with a low overpotential for HER. We hypothesize that the pH dependence is stronger for HER than for the acetoin reduction. The concentration of acetoin is significantly larger than the proton concentration, which leads to an increased acetoin reduction compared to HER. The impact of trace metals promoting HER seems less pronounced at the higher pH. This enables an enhanced conversion of acetoin in the FB at elevated pH values. The selectivity towards MEK in the FB is again higher than in the MS. Hereby, the high selectivity appears independent of the current acetoin concentration and remains more or less constant over the course of the experiment. HER increases with decreasing acetoin concentration and the FE drops from nearly 80 % after 15 minutes to 47 % at the  $t_{TC}$  and further to 25 % after 240 minutes. The course of selectivity, conversion, yield and FE for the reduction in the FB at a pH of 6.5 on lead is displayed in the Supporting Information in Figure S3. At  $t_{TC}$ , the conversion of acetoin is lower with 58 % in the FB compared to 89 % in the MS on lead. Next to the pH, this could be further tackled by the adjustment of other reaction parameters such as a lower current density for example or better mixing by an increased flow rate. A removal of metal ions via ion-exchange or electrodeposition could also prevent HER due to the elimination of active sites from trace components. Nevertheless, with a not yet optimized process resulting in a yield of 47 % and a high selectivity of 78 %, these results demonstrate the feasibility of the electrolysis directly in the fermentation broth.

### **Prolonged Experiments**

Experiments with the MS with acetoin concentration in the range of the FB were additionally conducted until acetoin was converted completely. The resulting concentrations are depicted in Figure 6.

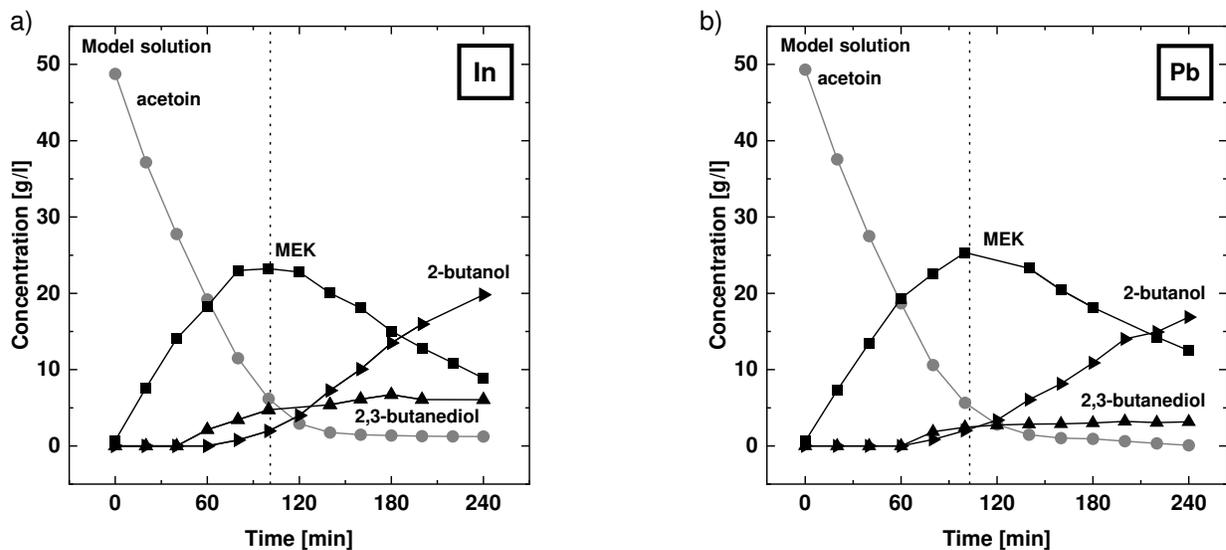


Figure 6: Course of concentration of reactants over the reaction time from the model solution on (a) lead and (b) indium at  $50 \text{ mA cm}^{-2}$ . Dashed vertical line indicates time of 100 % theoretical conversion ( $t_{TC}$ ).

When prolonging the experiment well beyond  $t_{TC}$ , the MEK concentration decreases and 2-butanol is formed. This appears slightly more pronounced on the indium electrode in Figure 6 a) than on lead in Figure 6 b). Both show a previously unreported electrochemical reduction of MEK to 2-butanol, rather than a direct reduction from acetoin to 2-butanol. In Figure 2 a) 2-butanol was also formed. However, at this low concentration, acetoin is depleted over the length of the electrochemical cell, which enables reduction of MEK to 2-butanol. No 2-butanol is apparent in experiments with the FB, as the lower overall conversion prevents the reduction of MEK because acetoin is not depleted during the experiment, even at lower initial concentration. The unwanted reduction of MEK could be prevented by continuous product removal. It could be achieved rather easily by gas-stripping, as MEK has a far higher vapor pressure at ambient conditions than acetoin, 2,3-butanediol or water. Alternatively, a lower current density and higher flow rate of the electrolyte could decrease the formation of 2-butanol until nearly all acetoin is depleted. However, a full conversion of acetoin at a reasonable current density would not be possible without continuous product removal.

## Conclusion and Outlook

In this work, we demonstrate a promising sustainable production route for MEK from glucose in a two-stage process: a fermentation with subsequent electrochemical reduction of the supernatant without further purification. We directly convert acetoin in the fermentation broth supernatant after the addition of a supporting electrolyte in an electrochemical cell. Therefore, we analyzed lead and indium for their activity in a model solution and a fermentation broth in LSV experiments. Then, we carried out lab-scale experiments setup with  $35\text{ cm}^2$  electrode area at  $50\text{ mA cm}^{-2}$  current density to quantitatively analyze the production of MEK. There, we analyzed the influence of the acetoin concentration in a model solution and by gradually decreased dilution of the fermentation broth. Reaction conditions that proved suitable for reduction in the model solution showed a poor yield in the fermentation broth. By adjusting the pH in the electrolysis cell to the pH of the fermentation broth, selective reduction was achieved with a yield and FE of FE,Y=47 %, and a selectivity of S= 79% from an undiluted fermentation broth on a lead electrode. While indium showed a better performance in model solution experiments, it showed a lower conversion and selectivity in experiments with the fermentation broth. On both electrodes, HER appeared to be the predominant competing reaction likely due to trace metal components in the complex fermentation medium. This detrimental effect could likely be reduced by removal of trace metals via electrodeposition or by altering the fermentation. Further, a previously unreported reduction from MEK to 2-butanol could be observed at low initial acetoin concentration and when acetoin was depleted in the electrolysis. In a future production process, this could be counteracted with a continuous gas stripping of MEK from the electrolyte. In future work, different acetoin producing organisms and the accompanying fermentation media should be investigated for an optimal interaction between fermentation and subsequent electrolysis. For the identified production route of MEK, a techno-economic assessment will reveal the most dominant bottlenecks.

## Acknowledgement

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## Supporting Information Available

The following files are available free of charge.

- Chromatogram of the fermentation broth supernatant as used for experiments in the fermentation broth. Table of the identified species and their retention time. Schematic reaction mechanism of acetoin to MEK and 2,3-BD as well as reduction of MEK to 2-butanol. Table with the composition of the Nakashimada medium, used for the fermentation. Course of selectivity, conversion, yield and FE over the experiment with the undiluted fermentation broth on lead at pH 6.5 and at pH 3.2 in the FB and the MS. SEM imaging and EDX mapping of electrodes after reduction in FB experiments.

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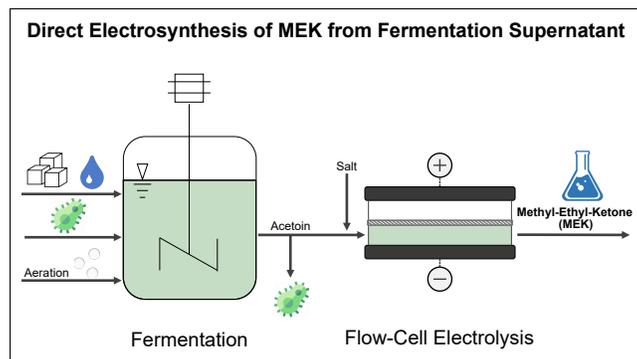
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## TOC Graphic



This work demonstrates a sustainable production process from glucose to methyl-ethyl-ketone (MEK) with the direct electroreduction of acetoin to MEK in a fermentation supernatant.

# SI: Direct Electrosynthesis of 2-Butanone from Fermentation Supernatant

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## Supporting Information

Table S1: Composition of Fermentation broth from Nakashimada<sup>?</sup>

S	Chemical	Chemical formula	Concentration [g/ml]
1	Glucose monohydrate	$C_6H_{12}O_6 \cdot H_2O$	0,6600000
2	Yeast extract		0,0500000
	Tryptone		0,0500000
3	Dipotassium hydrogen phosphate	$K_2HPO_4$	0,0175000
	Potassium dihydrogen phosphate	$KH_2PO_4$	0,0137500
	MES		0,0488000
4	Ammonium sulfate	$(NH_4)_2SO_4$	0,0055556
	Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	0,0013889
	Sodium molybdate dihydrate	$Na_2MoO_4 \cdot 2H_2O$	0,0006667
	Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0,0001167
5	Cobalt (II) nitrate hexahydrate	$Co(NO_3)_2 \cdot 6H_2O$	0,0029000
	Ammonium iron(II) sulfate hexahydrate	$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$	0,0039000
6	Nicotinic acid	$C_6H_5NO_2$	0,0002000
	Sodium selenite	$Na_2SeO_3$	0,0000172
	Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	0,0000053
	Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0,0005000
	Boric acid	$H_3BO_3$	0,0001000
	Potassium aluminium sulfate dodecahydrate	$AlK(SO_4)_2 \cdot 12H_2O$	0,0000172
	Copper (II) chloride dihydrate	$CuCl_2 \cdot 2H_2O$	0,0000010
	EDTA disodium dihydrate	$Na_2EDTA \cdot 2H_2O$	0,0005540

Table S2: Components and their retention times of the fermentation broth determined by HPLC.

Substance	Retention time [min]	Concentration [g/L]
Acetoin	22.92	48.3
2,3-Butanediol 1	23.85	
2,3-Butanediol 2	25	27.2
MEK	38.07	1.3
2-Butanol	46.2	0

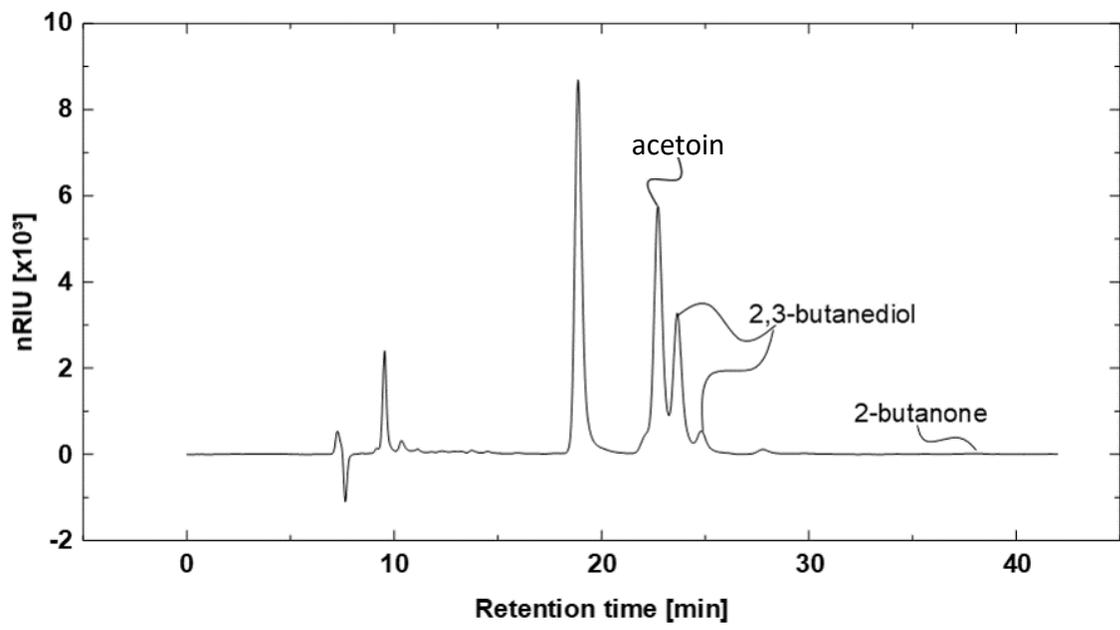


Figure S1: Chromatogram of fermentation broth supernatant

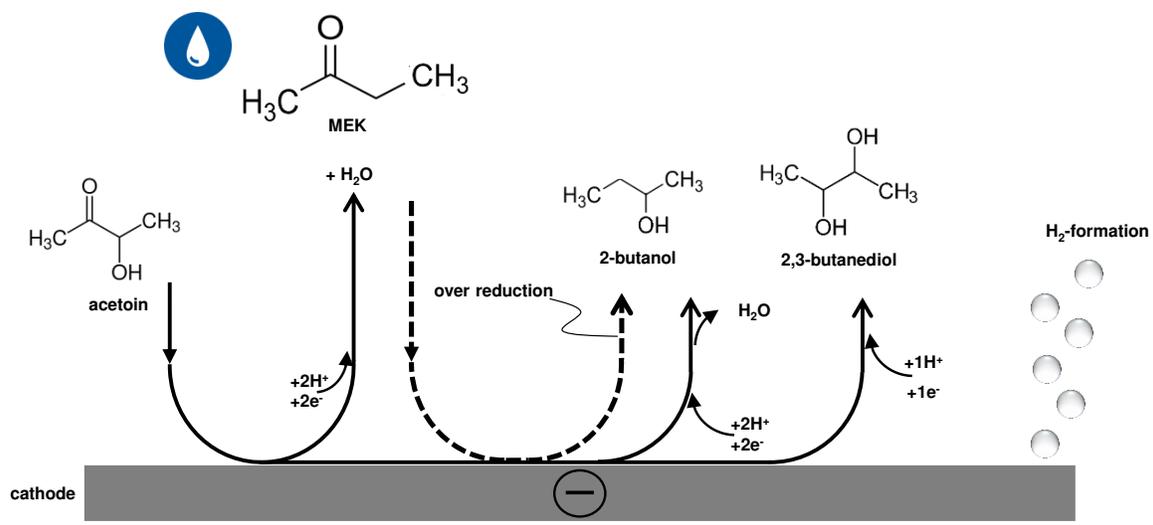


Figure S2: Reaction mechanism from acetoin to 2-butanone with 2,3-BD as side product and further reduction from 2-butanone to 2-butanol

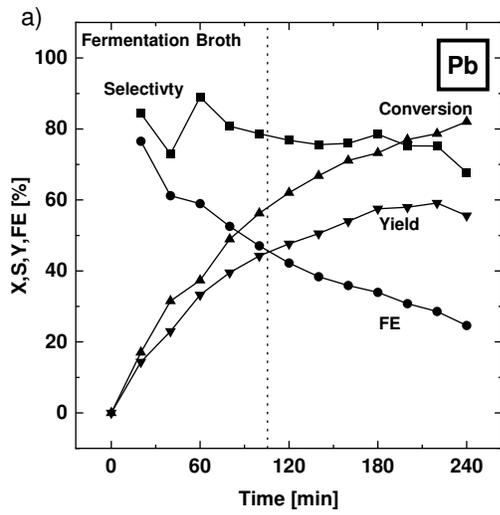


Figure S3: Selectivity, yield, FE and conversion over the course of the experiment of the FB at pH 6.5 on lead.

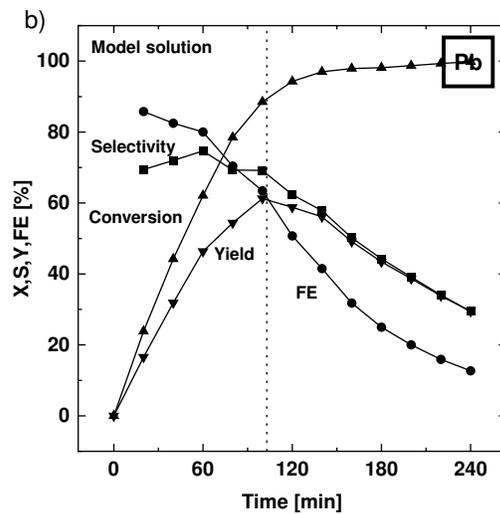
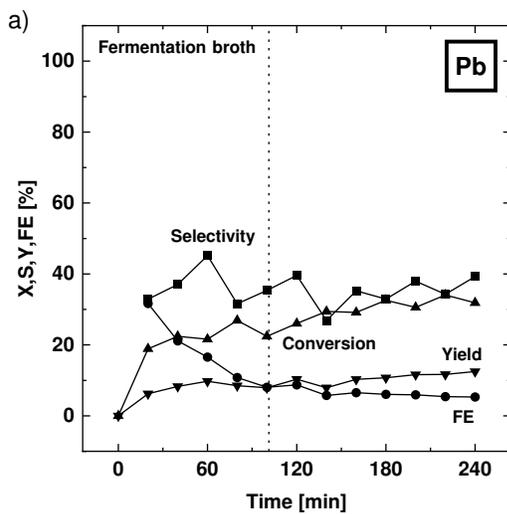


Figure S4: Selectivity, yield, FE and conversion over the course of the experiment on lead at pH 3.2 for a) the fermentation broth and b) the model solution.

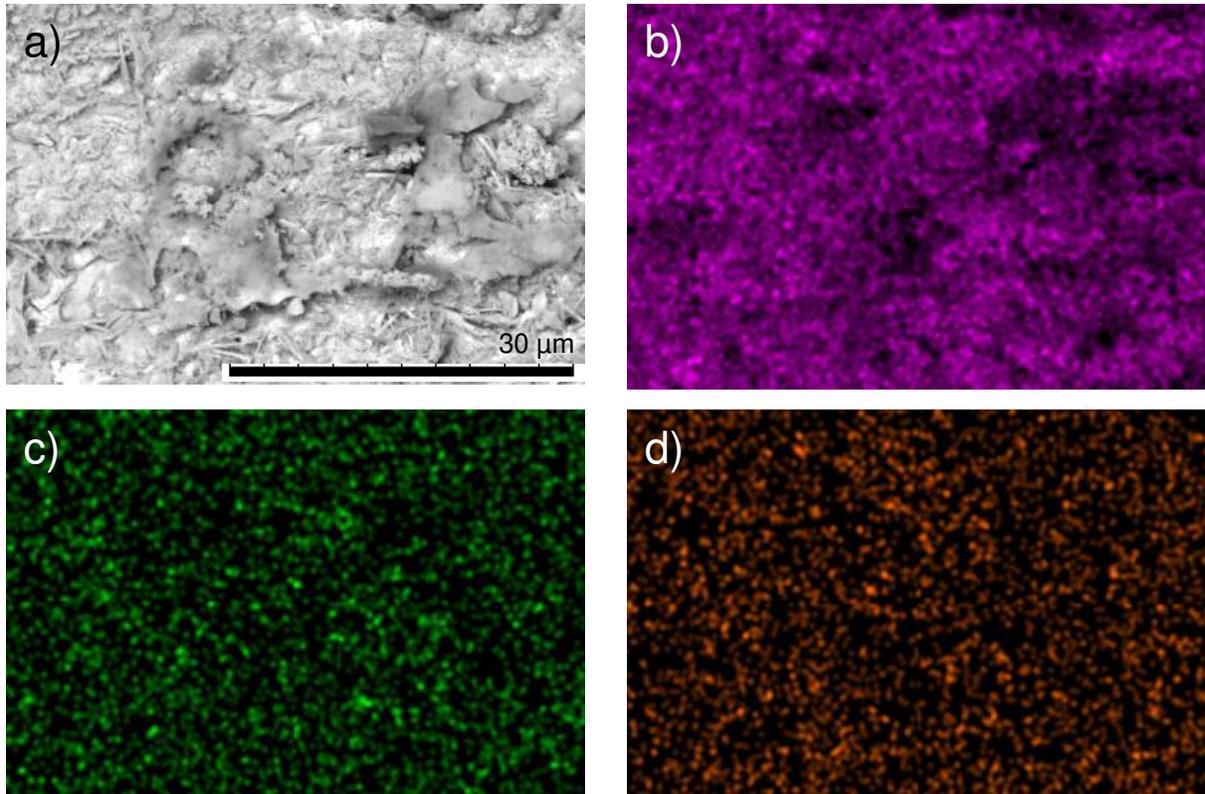


Figure S5: a) SEM and b-d) EDX analysis for b) lead, c) zinc and d) copper of the lead electrode after reduction in the undiluted FB