Aheterogeneous microbial consortium producing short chain fatty acids from lignocellulose

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Abstract:

Microbial consortia are a promising alternative to monocultures of genetically modified microorganisms for complex biotransformations. In this work, a versatile consortium-based strategy for the direct conversion of lignocellulose to short chain fatty acids was developed which included the funneling of the lignocellulosic carbohydrates to lactate as a central intermediate in engineered food chains. A spatial niche enabled *in situ* cellulolytic enzyme production by an aerobic fungus next to facultative anaerobic lactic acid bacteria and the product forming anaerobes. *Clostridium tyrobutyricum*, *Veillonella criceti* or *Megasphaera*

elsdenii were integrated into the lactate platform to produce e.g. 196 kg butyric acid per ton of beechwood. The lactate platform demonstrated the benefits of mixed cultures such as their modularity and their ability to convert complex substrates into valuable biochemicals.

One Sentence Summary: Microbial communities were engineered to funnel carbohydrates to lactate as an intermediate in synthetic food chains producing carboxylic acids.

Main Text:

Lignocellulosic biomass is an abundant source of fixed, renewable carbon and is a promising alternative to fossil petroleum to produce a range of chemicals. Short chain fatty acids (SCFAs) with two to six carbon atoms are currently produced from fossil resources and have been suggested as potential bio-derived platform molecules (*1*). However, the combination of the heterogeneous carbohydrate composition of lignocellulose and the limited metabolic flexibility of many SCFA producers leads to low product yields and undesirable by-product formation (**Fig. S1A)**. In order to provide a more homogeneous carbon source for SCFA producers, the heterogeneous mixture of carbohydrates in the biomass can be metabolically funneled to a central platform molecule in a primary fermentation followed by a secondary conversion to the desired product (**Fig. S1B**). An ideal system would combine the primary and secondary fermentation, as well as the depolymerization of the carbohydrates by cellulolytic enzymes, in one processing step termed consolidated bioprocessing (CBP) (*2*). The engineering of such complex reaction cascades into a single microbial host can create a substantial metabolic burden that limits the overall productivity of the system (*3*). Consequently, low carbon fluxes remain a major challenge for CBP approaches based on genetically engineered microorganisms (*4*, *5*), which are often characterized by metabolic imbalances (*6*–*8*). This limitation could be overcome by a metabolic division of labor (*3*) which is also observed in natural consortia, where complex reaction cascades are common and biochemically difficult tasks are catalyzed by multiple species according to the principle of metabolic compartmentalization (*9*). Each member of the community occupies its ecological niche and is specialized in a subtask or exchanges different beneficial molecules such as vitamins or amino acids. However, the high complexity of natural consortia makes the targeted engineering of the desired trait difficult and limits their industrial application for the production of biochemicals. Alternatively, less complex and more defined synthetic consortia can be engineered (*10*) and have been applied for CBP of lignocellulose to produce isobutanol

(*11*), ethanol (*12*, *13*), or acetone, butanol, and ethanol (*14*). A major challenge in the design of such bottom-up consortia is the requirement of overlapping tolerance ranges of several abiotic process parameters such as oxygen concentration, pH, and temperature, which limits the selection of possible consortium members (*15*). Thus, the majority of co-culture studies reported to date were carried out using only two species (*10*) with similar abiotic requirements and only few examples of artificial consortia with several populations have been reported (*16*–*19*).

Furthermore, under homogenous growth conditions, population control and achieving stable reproducible performance is challenging, especially for microbial consortia where mutual beneficial interactions are missing (*9*, *20*, *21*), for example, if one strain secretes cellulolytic enzymes to provide carbohydrates as a public good (*22*). In such cooperator/cheater communities, different growth rates of consortium members may lead to overgrowth of one population and extinction of the other (*23*). In order to tackle these challenges, we proposed to switch from a homogenous to a heterogenous reactor system that provides defined spatial niches where other abiotic conditions (e.g. oxygen, temperature, light) - fitted to one consortium member - prevail than in the otherwise homogeneous reactor environment (*24*). This enforced niche partitioning can stabilize microbial communities of e.g. an aerobe and facultative anaerobes (*24*–*27*) and enables the co-cultivation of microorganisms with nonoverlapping abiotic requirements.

Here, we demonstrate the feasibility of engineering spatial and metabolic niches as a strategy to build and control artificial cross-kingdom microbial consortia and introduce a CBP strategy termed “lactate platform” where lactic acid is used as a central and versatile intermediate for the direct production of different SCFAs from lignocellulose

Results

Many SCFA producers are non-cellulolytic strains of bacteria with limited flexibility to utilize different carbohydrates, but with the ability to metabolize lactic acid. This ability makes lactic acid a potential central intermediate in a microbial consortium-based CBP strategy aiming at the conversion of the complete carbohydrate fraction of lignocellulose to the target product (**Fig. S1B**). The lactate platform consists of: (i) the aerobic fungus *T. reesei* for the cooperative secretion of cellulolytic enzymes, which provide soluble sugars, (ii) facultative anaerobic lactic acid bacteria (LAB) that are able to convert all types of solubilized sugars to lactic acid and (iii) the lactate fermenting anaerobic product forming strain (**Fig. 1A**). In contrast to other abiotic factors such as temperature or pH (**Fig. 1B**), the oxygen requirements of the members of the artificial consortium do not overlap (**Fig. 1C**). By employing a membrane-aerated reactor in combination with the facultative anaerobic LAB, an oxygen gradient is generated in the reactor that allows fulfilling the redox potential requirements of all strains (**Fig. 1D**). To build the consortia, we followed a three-step inoculation scheme: First, the reactor was inoculated with *T. reesei* to allow the formation of a fungal biofilm on top of the membrane (**Fig. 1E**, **Fig. S2**). Secondly, after 48 h, the LAB was inoculated (designated as phase I in the figures with the time set to t = 0 h) and thirdly followed by the subsequent inoculation of the lactate fermenting strain(s) after waiting another 48 h (beginning of phase II). All insoluble substrates were added to the reactor at the time of the fungal inoculation, and soluble substrates were fed to the reactor after inoculation with LAB.

Butyric acid production

In order to produce butyric acid, a synthetic consortium consisting of *T. reesei*, *L. pentosus* for lactic acid production from glucose and xylose, and *C. tyrobutyricum* for producing the target product was set up. *C. tyrobutyricum* is able to convert glucose, xylose or a mixture of lactic and acetic acid to butyric acid (**Fig. 1A**, **Tab. S1)**. We first used a mixture of cellulose

(17.5 gL^{-1}) and xylose (9.32 gL^{-1}), which represented a model substrate for lignocellulose (**Fig. 2A**). The metabolic activity of *T. reesei* reduced the dissolved oxygen content below the detection limit within eight to twelve hours after fungal inoculation and lowered the redox potential to values between +20 and -20 mV (**Fig. S3**), which is not suitable for obligate anaerobes. Consequently, the co-cultivation of *T. reesei* and the obligate anaerobe *C. tyrobutyricum* was not possible (**Fig. S4**). Upon inoculation of *L. pentosus*, the redox potential dropped to less than -300 mV by reduction of oxygen to either hydrogen peroxide or water for the regeneration of NAD^+ by NADH oxidases (**Tab. S1**) (*28*), thereby creating suitable growth conditions for *C. tyrobutyricum*. Butyric acid production began after 100 h and was accompanied by a decrease of lactic and acetic acid. A maximum concentration of 4.1 gL^{-1} (0.14 gg^{-1} fermentable sugars, 93.1 % selectivity, 0.02 g total SCFA $L^{-1}h^{-1}$) was reached at the end of phase II (**Tab. S2**). At the beginning of phase III, lactic acid utilization by *C. tyrobutyricum* stopped; presumably because lactic acid cannot serve as a sole carbon source but must be co-consumed with either glucose or acetic acid (**Fig. S5A-E**) (*29*). To verify this hypothesis, 2 gL^{-1} acetic acid was fed as additional carbon source. Indeed, this enabled the complete utilization of lactic acid to produce a concentration of 10.7 gL^{-1} butyric acid (0.37 gg^{-1}, 77.6 % selectivity, 0.05 g total SCFAs $L^{-1}h^{-1}$, **Fig. 2B, Tab. S2**).

As an alternative to the addition of acetic acid, we aimed to enhance the metabolic flux from carbohydrates to acetic acid by extending the microbial consortium with the obligate heterofermentative strain *Lactobacillus brevis* which produces lactic and acetic acid from both hexoses and pentoses via the phosphoketolase (PK) pathway (**Tab. S1**). In comparison with the base case consortium (**Fig. 2A**), the extended microbial consortium produced a higher amount of acetic acid in phase I and II (**Fig. 2C**). This enabled the complete re-utilization of lactic acid leading to 9.5 gL^{-1} butyric acid (0.33 gg^{-1}, 77.9 % selectivity, 0.06 g total SCFAs $L^{-1}h^{-1}$, **Tab. S2**). The lower butyric acid selectivity compared to the base case is due to the slight overproduction of acetic acid.

Finally, we tested elevating the acetic acid concentration in the system by minimizing the its previously reported degradation by *T. reesei* (*26*), which could be limited by the amount of oxygen entering the reactor. Indeed, decreasing the oxygen transfer rate (OTR) by a factor of about ten by using a siloxane membrane with a thicker wall led to a higher concentration of accumulated acetic acid in phase II (**Fig. 2D**). Then, lactic acid could be completely converted to 10.2 gL^{-1} butyric acid after 372 hours (0.35 gg^{-1}, 96.7 % selectivity, 0.03 g total SCFAs $L^{-1}h^{-1}$, **Tab. S2**).

In order to obtain a benchmark for conventional butyric acid production, we also performed simultaneous saccharification and fermentation (SSF) experiments with the same substrates using only *C. tyrobutyricum* and a celluloytic enzyme cocktail. A yield of 0.34 gg^{-1} butyric acid with a selectivity of 82 % (**Fig. S7**) was achieved, which is slightly lower than the corresponding results of the lactate platform (**Tab. S2**).

Influence of substrate composition on the theoretical butyric acid yields

In order to evaluate and compare the different options for producing butyric acid from lignocellulosic biomass and model substrates, we calculated the theoretical yields as a function of the substrate composition and the chosen fermentation route. To determine the theoretical yields of the lactate platform we assumed that all hexoses are fermented by *L. pentosus* through the Embden-Meyerhof-Parnas pathway to lactic acid although *C. tyrobutyricum* is also able to consume glucose. This assumption is justified as *L. pentosus* – in contrast to *C. tyrobutyricum* – can use cellobiose intracellularly which favors the metabolic flux of hexoses to lactic acid (**Fig. 1A**) (*30*). Furthermore, the cessation of lactic acid consumption by *C. tyrobutyricum* upon acetic acid depletion observed in the above presented experiments (**Fig. 2A**) is a strong indication that no glucose is available for this strain, as lactic acid can also be metabolized in the presence of glucose (**Fig. S5F-J**). We also assume that xylose is metabolized through the PK pathway to lactic and acetic acid solely by *L.*

pentosus (**Tab. S1**). These products as well as the acetic acid present in the feedstock are then converted by *C. tyrobutyricum* to butyric acid. We experimentally determined the required ratio of lactic to acetic acid and found that 3 moles of lactic acid and 1 mol of acetic acid were converted to 2 moles of butyric acid (**Fig. S5A-E**) which is in line with literature data (*29*). In **Fig. S6** we propose the biochemical basis of the lactate/acetate co-fermenting pathway to butyric acid and compare it to the glucose fermentation to butyric acid in *C. tyrobutyricum*. The yield based on the mass of fermentable carbohydrates for direct sugar conversion varies only slightly between 0.486 gg^{-1} for glucose and 0.493 gg^{-1} for xylose; however it ranges from 0 gg^{-1} (for pure glucan) up to 0.635 gg^{-1} (81 % glucan, 19 % acetic acid) for the lactate platform (**Fig. 2E**). As acetic acid is typically an unused resource in real biomass, only the carbohydrates were counted as carbon source. If acetic acid is counted as part of the substrate, the maximal theoretical butyric acid yield of the lactate platform is 0.52 gg^{-1} carbon source which is 7 % higher than the theoretical yield of the direct sugar conversion. For a feedstock composition corresponding to that of pretreated beechwood (68.5 % glucan, 24.3 % xylan, 7.2 % acetic acid), the theoretical yield of butyric acid produced via the lactate platform is 0.525 gg^{-1} fermentable carbohydrates compared to 0.488 gg^{-1} for the direct fermentation of the carbohydrates. To underpin this theoretical framework with experimental data, we determined the butyric acid yield of the lactate platform using the optimal mixture of glucose and acetic acid. To this end, glucose was first metabolized to lactic acid using *L. pentosus*, followed by the addition of *C. tyrobutyricum* and acetic acid in the second phase of the fermentation. A butyric acid yield of 0.53 gg^{-1} glucose or 0.43 gg^{-1} carbon source, respectively, could be achieved, which corresponds to 109.4 ± 2.7 % of the theoretical yield for the direct glucose conversion by *C. tyrobutyricum* (**Fig. 2F, G**). This result also outperforms published yields for fermentations of the same substrates employing natural or genetically engineered strains (**Tab. S4**).

Propionic and acetic acid production

To demonstrate the modularity of the lactate platform we replaced *C. tyrobutyricum* with *V. criceti* to produce propionic acid. *V. criceti* is an obligate anaerobic bacterium which is unable to utilize carbohydrates but converts lactic acid to propionic and acetic acid (**Fig. 1A, Tab. S3**). Thus, the lactate platform approach compensates for the missing metabolic flexibility of the strain and enables the production of propionic acid from carbohydrates. CBP of 17.5 gL^{-1} microcrystalline cellulose and 9.32 gL^{-1} xylose yielded 8.9 gL^{-1} total carboxylic acids (**Fig. S8**, **Tab. S5**). When doubling both carbon sources, 13.7 gL^{-1} acetic acid and 9.3 gL^{-1} propionic acid were produced (**Fig. 3A, Tab. S5**).

Mixed C_2 to C_6 SCFA production

Higher SCFAs such as valeric (C_5) and caproic (C_6) acid are more valuable than the lower SCFAs (C_2 to C_4). However, only few microbial species are known to produce these compounds in substantial amounts (*31*). One of the exceptions is *Megasphaera elsdenii*, a strict anaerobic rumen bacterium. *M. elsdenii* favors lactic acid as carbon source compared to glucose, and is not capable to metabolize xylose or cellulose (*32*, *33*), thereby making it a suitable candidate for the lactate platform (**Fig. 1A**). We tested the co-cultivation of *T. reesei*, *L. pentosus* and *M. elsdenii* in the membrane-aerated reactor following the subsequent inoculation strategy described above. With 17.5 gL^{-1} cellulose and 9.3 gL^{-1} xylose as substrates, we recorded the simultaneous accumulation of 4.3 gL^{-1} acetic acid, 1.2 gL^{-1} propionic acid, 5.0 gL^{-1} butyric acid, 1.5 gL^{-1} valeric and 1.3 gL^{-1} caproic acid in 280 h. The obtained SCFA yields were 0.46 ± 0.01 g SCFA per g fermentable sugars (**Fig. 3C, Tab. S6**) showing the successful co-cultivation of the three strains. However, after inoculation of *M. elsdenii* the redox potential increased (around 1.5 mVh^{-1} from t=50 h to t=200 h) indicating that the metabolic activity of *T. reesei* and *L. pentosus* was not sufficient to maintain a sufficiently low redox potential in longer fermentation runs, likely due to product toxicity.

CBP of pretreated beechwood

After successfully converting model substrates, we targeted the direct conversion of a lignocellulosic substrate - pretreated beechwood - to the different target products. We applied a two-stage steam pretreatment procedure to recover temperature sensitive solubilized xylooligomers and acetic acid in a moderately inhibitory liquid stream termed prehydrolyzate after a mild first pretreatment and a well digestible solid substrate after the second more severe pretreatment step (**Fig. 4**, **Fig. S9**). For CBP of beechwood, we added 3 % (w/w) pretreated solids at the beginning of the fermentation and started the first stage prehydrolyzate feed with the inoculation of *L. pentosus*. With *C. tyrobutyricum* as lactic acid fermenting strain, the consortium produced 9.5 gL^{-1} butyric acid (0.38 gg^{-1} fermentable carbohydrates, 91.5 % selectivity, 0.014 g total SCFA $L^{-1}h^{-1}$, **Fig. S10A**, **Tab. S2**), which corresponds to an overall yield of 196.5 kg butyric acid per ton of dry raw beechwood (**Fig. 4B**). This yield was approximately twice as high as the reported yields of a natural consortium (*34*), an anaerobic co-culture (*35*) or a natural single cellulolytic strain (*36*). They were even comparable to yields achieved on lignocellulosic hydrolysates (*37, 38*) although we did not add external enzymes (**Tab. S4**). When *V. criceti* was used as fermenting strain in the lactate platform, 6.7 gL^{-1} acetic acid and 5.6 gL^{-1} propionic acid (0.49 gg^{-1} fermentable carbohydrates, 0.031 g total SCFA $L^{-1}h^{-1}$, **Fig. S10B**, **Tab. S5**) were produced. This corresponded to 133.3 kg acetic acid and 113.6 kg propionic acid per ton of raw biomass (**Fig. 4B**). Finally, when *M. elsdenii* was integrated into the lactate platform, a mixture of 6.8 gL^{-1} of acetic acid, 4.2 gL^{-1} of butyric acid, 2.4 gL^{-1} of caproic acid and small amounts of propionic and valeric acid (each around 1 gL^{-1}) (**Fig. S10C, Tab. S6**) could be produced from pretreated beechwood. Overall, the process yielded 0.42 g total SCFAs per g total fermentable sugars corresponding to 217.1 kg total SCFAs per ton of raw beechwood with a productivity of 0.012 $gL^{-1}h^{-1}$. Even after more than 600 hours after inoculation of *M. elsdenii*, the accumulation of metabolic end-products of the anaerobic pathways such as caproic acid was recorded indicating that the

reduced oxygen transfer rate achieved by using a thicker membrane prolonged the metabolic activity of the strict anaerobic strain (**Fig. S10C, Tab. S6**). This is in line with the measured smaller slope of the redox potential (around 0.23 mVh^{-1} from t=400 h to t=1094 h) in comparison to the approach with the thinner membrane (**Fig. 3B**). CBP of lignocellulosic substrates to mixed SCFAs can also be performed by natural mixed cultures such as the rumen microbiome or different kind of anaerobic digestion consortia, where methane formation is inhibited. These processes are summarized as the "carboxylate platform" (*1*) and are typically performed with undefined waste substrates making a comparison of the yields with the lactate platform difficult. However, the yield that we achieved with the lactate platform is comparable to reported *M. elsdenii* fermentations on glucose or corn stover hydrolysate, while we even exceeded the reported titers (**Tab. S4**). Furthermore, in the lactate platform xylose was also converted, which is not possible in axenic *M. elsdenii* cultures.
In the batches run on beechwood, we also quantified hydrolytic enzyme expression over time to determine whether *T. reesei* was metabolically active and was producing enzymes during all phases of the fermentations. We consistently found increasing activities of cellobiohydrolases, endoglucanases, beta-glucosidases and xylanases in the supernatants including after inoculation with the lactic acid and product forming strains (**Fig. S11**). Together with further experiments described in the SM (**Fig. S18 - S20**), we conclude that *T. reesei* was metabolically active throughout the experiments.

Extension of the lactate platform to four members

Finally, we aimed to influence the share of odd- or even-numbered SCFAs that were produced based on the analysis of metabolic pathways of *M. elsdenii* (**Fig. 1A**). *M. elsdenii* can take up extracellular propionic acid which is activated intracellularly to propionyl-CoA by a propionyl-CoA transferase (PCT) (**Fig. 1A**). Due to the broad substrate spectrum of the PCT the functional group CoA can be swapped between acetyl-CoA and SCFA (*39*, *40*), which is

essential for the chain elongation process. Based on this, we hypothesized that an increased availability of propionic acid increases the production of higher, odd-numbered SCFAs. As *V. criceti* was shown to convert lactic acid to propionic and acetic acid, thereby increasing the concentration of propionic acid, we set up a lactate platform containing both *V. criceti* and *M. elsdenii* as lactate fermenting strains. Using pretreated beechwood as substrate, this artificial consortium produced 2.6 gL^{-1} of acetic acid, 0.8 gL^{-1} of propionic acid, 6.7 gL^{-1} butyric acid, 3.5 gL^{-1} valeric acid and 2.1 gL^{-1} of caproic acid (**Fig. 4B, Fig. S10E**). Thus, the valeric acid concentration is almost by a factor of three higher as compared to the mixture produced by the community without *V. criceti*. The process yield is with 0.42 g total SCFAs per g total fermentable sugars and 0.22 g total SCFAs per g of raw beechwood similar to the community without *V. criceti*. The metabolism of *M. elsdenii* also allows the reverse beta-oxidation of butyric acid (electron acceptor) taken from the environment to caproic acid if an electron donor such as lactate is available (**Fig. 1A**). Thus, by extending the community with *C. tyrobutyricum* instead of *V. criceti* we targeted to increase the share of even-numbered longer SCFA. By doing so, we measured a SCFA mixture consisting of 4.6 gL^{-1} of acetic acid, 1.0 gL^{-1} of propionic acid, 7.4 gL^{-1} butyric acid, 1.4 gL^{-1} valeric acid and 2.5 gL^{-1} of caproic acid (**Fig. 4B, Fig. S10D**). Compared to the base case, the butyric acid concentration increased by a factor of 1.75 and the acetic acid concentration decreased (factor 0.66). However, the presence of *C. tyrobutyricum* did not increase the caproic acid concentration which indicates the lack of electron donors that are required for chain elongation by *M. elsdenii* (*41*). Overall, the yield achieved with the community extended with *C. tyrobutyricum* increased to 0.46 g total SCFAs per g total fermentable sugars and 0.24 g SCFAs per g raw beechwood.

Discussion and outlook

In this study, we merged synthetic microbial ecology with process engineering to realize and control artificial food chains distributed over up to four highly diverse microorganisms. Our goal was to provide a platform that minimizes the required metabolic capabilities of product forming microorganisms to facilitate their integration into an artificial community utilizing all biomass fractions. To demonstrate the platform's wide applicability, we identified several other bulk or fine chemicals including acetic acid, different alcohols (ethanol, propanol, butanol, 1,2-propanediol), polyhydroxybutyrate and lipids that could be produced through the lactate platform (**Fig. S12**). We analyzed the stoichiometries of the required pathways and found that the lactate platform theoretically boosts the carbon flux from lignocellulose to the target product compared to the alternative direct sugar fermentation routes for acetic acid, polyhydroxybutyrate, ethanol and butanol (**Fig. S12, Tab. S7 - S9**). A switch to another target product may require the exchange of the lactate fermenting strain(s) and with that, different abiotic requirements such as pH, temperature or presence of co-substrates will likely have to be fulfilled. Furthermore, for some products the artificial food chain must be extended, *i.e.* consortia containing at least four different microorganisms would be necessary. Thus, the creation of additional spatial and/or metabolic niches is desirable to fully harness the potential of the lactate platform. To this end, we proposed several possibilities to adapt the biofilm membrane reactor (*24*). For instance, temperature gradients could be created by installing local heat sinks or sources in the reactor or pH gradients could be formed by introducing carbon dioxide or ammonia through a membrane to the reactor (**Fig. S13**). In the same way, co-substrates such as hydrogen or carbon monoxide could be fed to the consortium. Furthermore, light management in the reactor could facilitate the mixotrophic growth of microalgae on lignocellulosic feedstocks which paves the way to a range of valuable products such as lipids for biodiesel production or omega-3 fatty acids as aquatic feed. As a first step in the direction of the latter, we showed that *T. reesei* and the microalgae *Chlamydomonas*

reinhardtii were able to grow together in an illuminated aerobic biofilm using pure cellulose as a substrate (**Fig. S14 - S17**). Prior to a potential industrial application further optimization of the lactate platform is necessary – especially the product concentrations and the corresponding productivities need to be enhanced. At this proof of concept stage, the obtained productivities in the range of 0.01 to 0.06 $gL^{-1}h^{-1}$ (**Tab. S2, Tab. S5 - S6**) compare well with literature data (**Tab. S4**). One reason for the moderate productivities is the gap between the optimal cellulase production temperature (28 – 30°C) and the optimal temperature for enzyme activity (40 to 50°C). Besides using an advanced industrial cellulase producing strain, the use of a temperature gradient reactor could also increase the productivity. The bulk phase could be operated at elevated temperatures, while the enzyme producing biofilm could be cooled via the membrane.

Taken together, the results achieved with the lactate platform demonstrate the inherent benefits of mixed artificial cultures such as their modularity and their ability to convert complex substrates to valuable platform chemicals. This reactor engineering complements the available toolbox for successfully engineering stable and controllable synthetic communities for numerous novel applications.

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Supplementary Materials:

Materials and Methods

Figures S1-S20

Tables S1-S9

References (*43-70*)

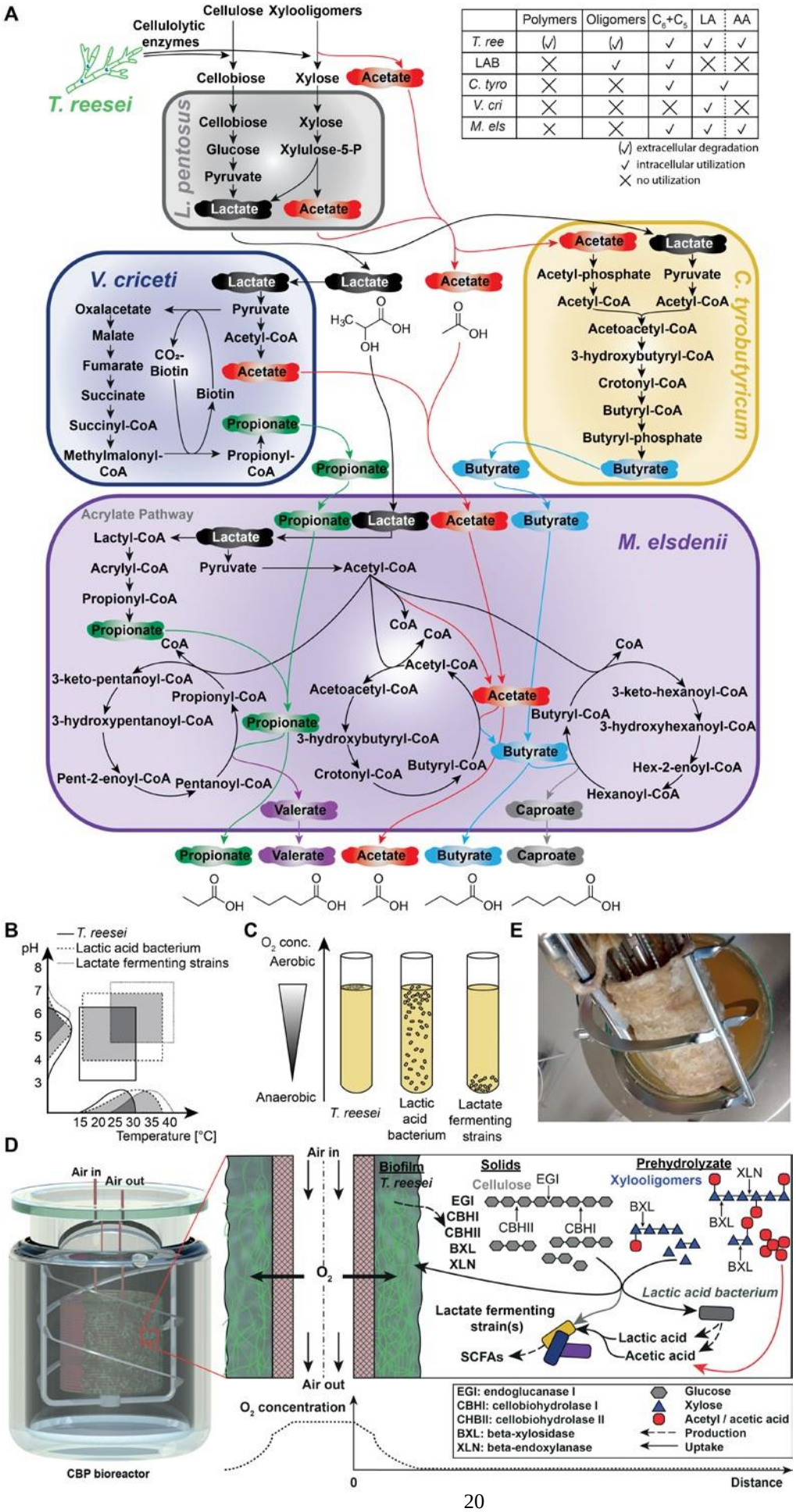
A
Cellulose Xylooligomers
Cellulolytic enzymes
T. reesei
Cellobiose
Xylose
Acetate
L. pentosus
Cellobiose
Xylose
Glucose
Xylulose-5-P
Pyruvate
Lactate
Acetate
Polymers
Oligomers
C6+C5
LA
AA
T. ree
LAB
C. tyro
V. cri
M. els
(√) extracellular degradation
√ intracellular utilization
× no utilization
V. criceti
Lactate
Oxalacetate
Pyruvate
Malate
Acetyl-CoA
Fumarate
CO2-Biotin
Acetate
Succinate
Biotin
Succinyl-CoA
Propionate
Methylmalonyl-CoA
Propionyl-CoA
C. tyrobutyricum
Acetyl-phosphate
Pyruvate
Acetyl-CoA
Acetoacetyl-CoA
3-hydroxybutyryl-CoA
Crotonyl-CoA
Butyryl-CoA
Butyryl-phosphate
Butyrate
M. elsdenii
Acrylate Pathway
Lactyl-CoA
Acrylyl-CoA
Propionyl-CoA
CoA
3-keto-pentanoyl-CoA
3-hydroxypentanoyl-CoA
Pent-2-enoyl-CoA
Pentanoyl-CoA
Valerate
Acetoacetyl-CoA
3-hydroxybutyryl-CoA
Crotonyl-CoA
Butyryl-CoA
3-keto-hexanoyl-CoA
3-hydroxyhexanoyl-CoA
Hex-2-enoyl-CoA
Hexanoyl-CoA
Caproate
B
T. reesei
Lactic acid bacterium
Lactate fermenting strains
pH
Temperature [°C]
C
O2 conc.
Aerobic
Anaerobic
T. reesei
Lactic acid bacterium
Lactate fermenting strains
E
D
Air in
Air out
CBP bioreactor
Biofilm
T. reesei
Solids
Cellulose
Prehydrolyzate
Xylooligomers
EGI
CBHI
CBHII
BXL
XLN
O2
Lactic acid bacterium
Lactate fermenting strain(s)
Lactic acid
Acetic acid
SCFAs
O2 concentration
Distance
EGI: endoglucanase I
CBHI: cellobiohydrolase I
CHBII: cellobiohydrolase II
BXL: beta-xylosidase
XLN: beta-endoxylanase
Glucose
Xylose
Acetyl / acetic acid
Production
Uptake

Fig. 1. Setup of the lactate platform. (A) Selected metabolic pathways in the community-based CBP using lactate and acetate as central intermediates. Table: Capability of strains to metabolize polymers (cellulose and hemicellulose), oligomers (gluco- and xylooligosaccharides), C_5 / C_6 sugars, lactate (LA) and acetate (AA). **(B)** Approximate tolerance ranges for pH and temperature of strains (*42*). **(C)** Requirements of strains for dissolved oxygen concentration. **(D)** Schematic representation of the membrane-aerated bioreactor for CBP of lignocellulosic biomass to SCFAs with dissolved oxygen concentration profile. **(E)** Photograph of mature fungal biofilm on the membrane.

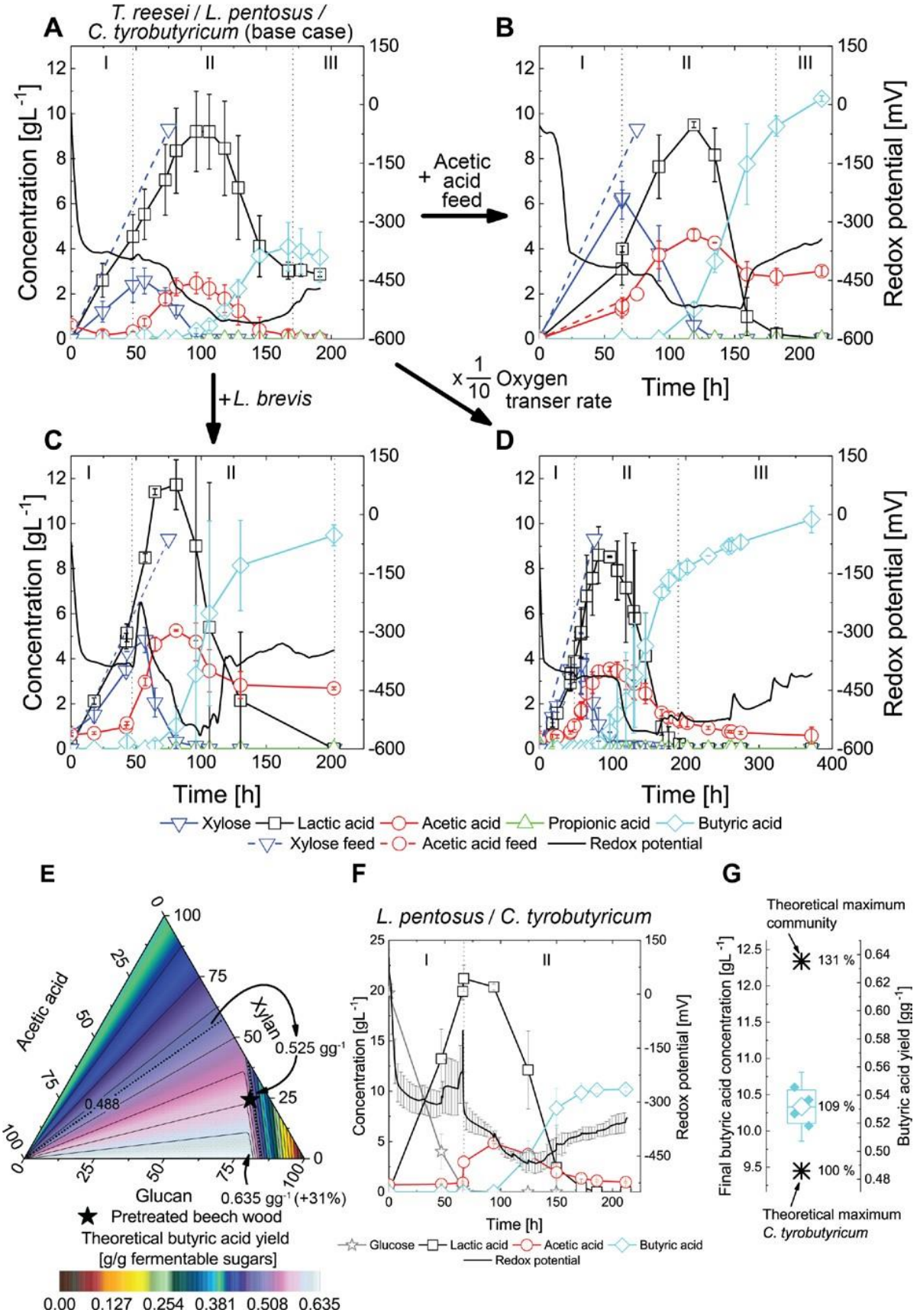
A
T. reesei / L. pentosus / C. tyrobutyricum (base case)
B
+ Acetic acid feed
+L. brevis
x 1/10 Oxygen transer rate
C
D
Concentration [gL-1]
Redox potential [mV]
Time [h]
I
II
III
Xylose
Lactic acid
Acetic acid
Propionic acid
Butyric acid
Xylose feed
Acetic acid feed
Redox potential
E
Acetic acid
Xylan
0.525 gg-1
0.488
Glucan
0.635 gg-1 (+31%)
Pretreated beech wood
Theoretical butyric acid yield
[g/g fermentable sugars]
0.00 0.127 0.254 0.381 0.508 0.635
F
L. pentosus / C. tyrobutyricum
Glucose
G
Theoretical maximum community
131 %
109 %
100 %
Theoretical maximum C. tyrobutyricum
Final butyric acid concentration [gL-1]
Butyric acid yield [gg-1]

Fig. 2. CBP of cellulose and xylose to butyric acid (A) In the base case, 17.5 gL^{-1} Avicel and 9.32 gL^{-1} xylose (fed over a time period of 75 h, dashed blue line) were converted to butyric acid by a consortium of *T. reesei*, *L. pentosus* and *C. tyrobutyricum* at an oxygen transfer rate (OTR) of 3.24 h^{-1}. Differences from the base case: **(B)** Acetic acid feed to 2 gL^{-1} over a time period of 75 h (dashed red), **(C)** addition of *L. brevis* to the consortium, **(D)** reduction of OTR to 0.34 h^{-1}. Error bars represent the standard deviation from two independent experiments. Stage III depicts the condition in which *C. tyrobutyricum* lacks acetic or lactic acid. **(E)** Ternary plot of theoretical butyric acid yield for feedstocks containing different fractions of glucan, xylan and acetic acid (further details in supplementary materials). **(F)** Two stage fermentation of the yield optimized glucose / acetate mixture. In stage I, glucose was fermented by *L. pentosus* to lactic acid followed by acetic acid addition and inoculation with *C. tyrobutyricum* in phase II. **(G)** Observed and theoretical butyric acid concentration and yield for the lactate platform. Error bars in **(A)-(D), (F)** represent the standard deviation of the mean. The box in **(G)** represents the standard deviation of the mean and error bars the 99 % confidence interval.

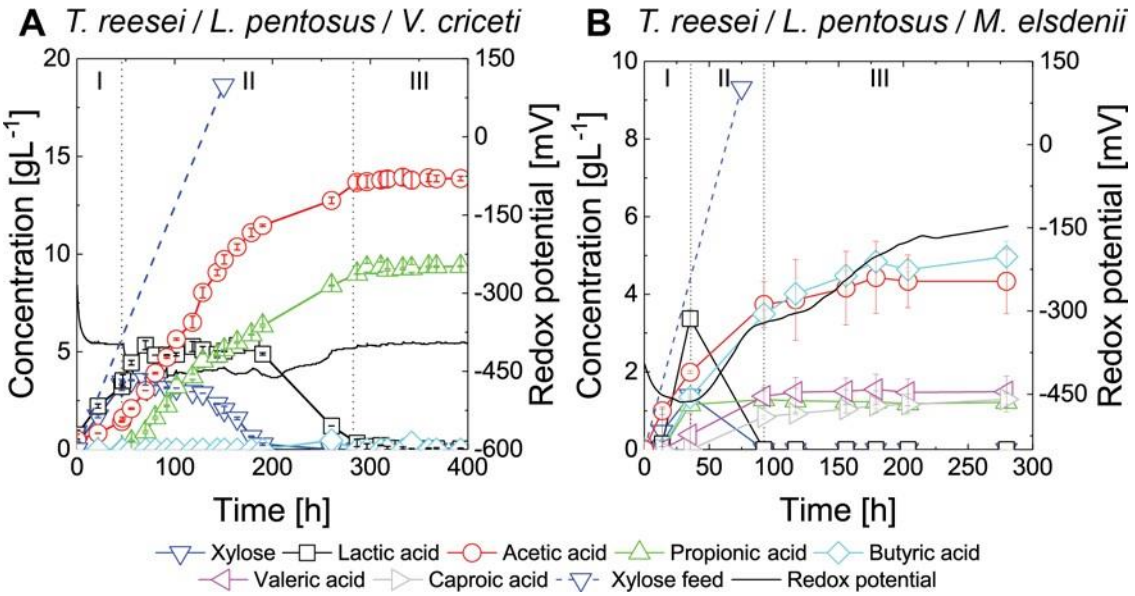

Fig. 3. CBP of cellulose and xylose to propionic and acetic acid or mixed SCFAs. (A) CBP of 35 gL^{-1} Avicel and 18.64 gL^{-1} xylose (fed over a time period of 150 hours) to propionic and acetic acid with *V. criceti* as lactate fermenting strain. **(B)** CBP of 17.5 gL^{-1} Avicel and 9.32 gL^{-1} xylose (fed over a time period of 75 hours) to mixed SCFAs with *M. elsdenii* as lactate fermenting strain. Stage III depicts the condition in which *V. criceti* or *M. elsdenii* lack lactic acid. Error bars represent the standard deviation from two independent experiments.

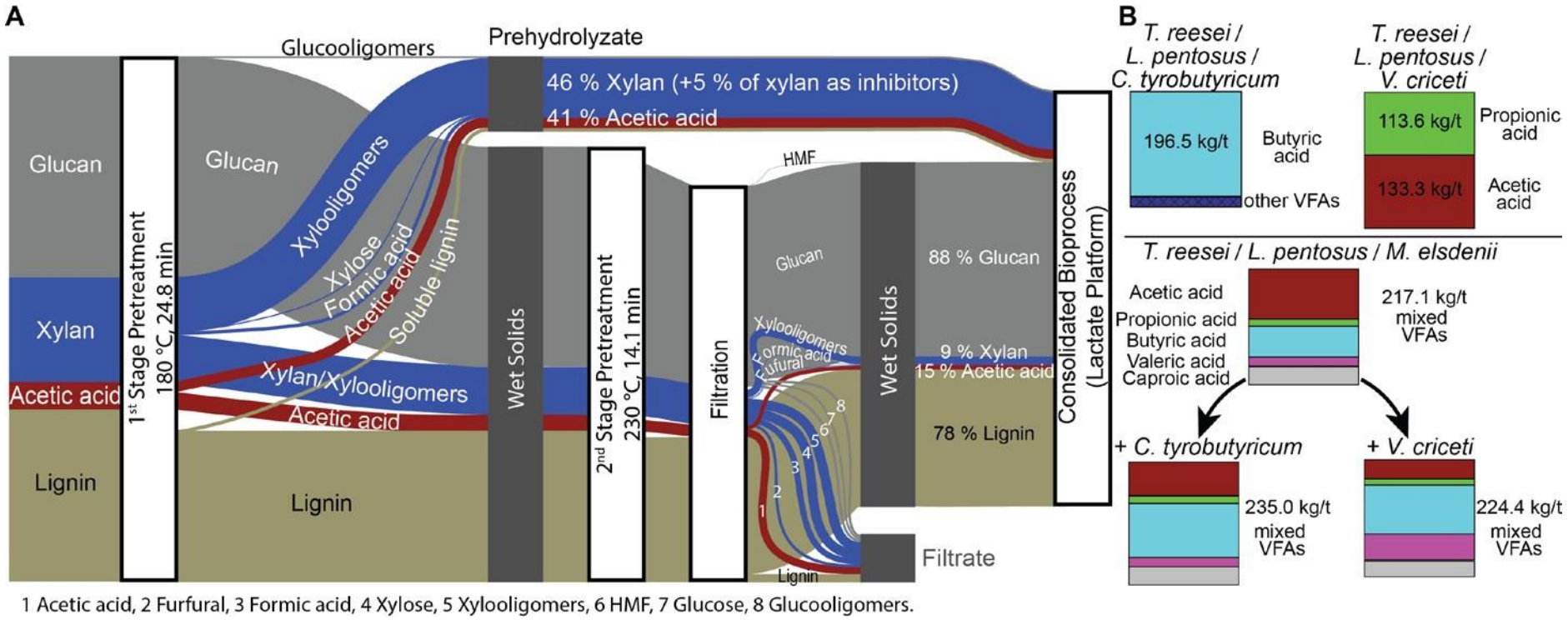

Fig. 4. Steam-pretreatment of beechwood and CBP to SCFAs. (A) Mass fluxes of glucan, xylan, acetic acid and lignin in the two-stage steam-pretreament (further details in supplementary materials**, Fig. S9**). **(B)** Experimental yields of CBP with different microbial consortia to various SCFAs (**Fig. S10**).

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Supplementary Materials for

A heterogeneous microbial consortium producing short chain fatty acids from lignocellulose

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This PDF file includes:

Materials and methods

Fungal, bacterial and algal strains and culturing methods

L. pentosus (DSM-20314), *L. brevis* (DSM-20054), *C. tyrobutyricum* (DSM-2637) *V. criceti* (DSM-20734), and *M. elsdenii* (DSM-20460) were purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

One mL of glycerol stock was inoculated to MRS medium for precultures of *Lactobacillus* in 500 mL Erlenmeyer flasks and incubated for 48 hours at 150 rpm at 33 °C. The MRS medium contained (gL^{-1}): peptone from casein, 10; meat extract, 10; yeast extract, 5; glucose, 20; Tween 80, 1; K_2HPO_4, 2; sodium acetate, 5; ammonium citrate, 2 and $MgSO_4 \cdot 7\ H_2O$, 0.2. The pH was adjusted to 6.2-6.5 with hydrochloric acid.

C. tyrobutyricum and *M. elsdenii* precultures were cultivated in a reinforced clostridial medium (RCM). Composition of the RCM medium (in gL^{-1}): yeast extract, 13; peptone, 10; glucose, 5; soluble starch, 1; sodium chloride, 5; sodium acetate, 3; L-cysteine-HCl, 0.5; agar, 0.5.

V. criceti precultures were cultivated in medium containing (in gL^{-1}): trypticase (BBL), 5; yeast extract, 3.0; Na-(DL)-lactate, 7.5; Na-thioglycolate, 0.75; Tween 80, 1.0; Glucose, 1.0; Na-resazurin solution (0.1 % (w/v)), 0.5 mL. The medium for *C. tyrobutyricum* and *V. criceti* was boiled under continuous flushing with nitrogen and the pH was adjusted to 6.8 before it was aliquoted to 100 mL serum bottles (50 mL in each) and autoclaved at 121 °C for 20 minutes.

V. criceti is known for its high nutrient requirements (*43*, *44*), but we could show, that it is still active on Mandels medium containing only 1.0 gL^{-1} complex media components. We found that the fermentation time was prolonged by a factor of about three without further supplementation of complex nutrients, while the yields remained the same (**Tab. S3**). Therefore, we used Mandel medium in the CBP experiments with *V. criceti*.

All serum bottles were inoculated from a two-day liquid culture stored at 4 °C and incubated on an orbital shaker at 140 rpm at 37 °C.

T. reesei Rut-C30 (ATCC 56765) was purchased from the VTT, Finland. Spores from a seven-day old potato dextrose agar slant culture were incubated at 28 °C and stored at 4 °C. For precultures, Mandels medium containing 7.5 gL^{-1} microcrystalline cellulose (Avicel PH-101, Sigma Aldrich, Buchs, Switzerland) was inoculated with spores from the agar slant in an Erlenmeyer flask for four days at 28 °C and 150 rpm. Mandels medium contained the following ingredients (gL^{-1}): KH_2PO_4, 2; $(NH_4)_2SO_4$, 1.4; $MgSO_4 \cdot 7\ H_2O$, 0.3; $CaCl_2 \cdot 6\ H_2O$, 0.4; urea, 0.3; peptone, 0.75; yeast extract, 0.25 and trace element stock, 1 mLL^{-1}. The trace element stock contained (gL^{-1}): $FeSO_4 \cdot 7\ H_2O$, 5; $MnSO_4 \cdot H_2O$, 1.6; $ZnSO_4 \cdot 7\ H_2O$, 1.4; $CoCl_2 \cdot 6H_2O$, 3.7 and 10 mLL^{-1} concentrated hydrochloric acid and was sterile filtered. To avoid precipitation, 100x $CaCl_2$ and $MgSO_4$ solutions were autoclaved separately before merging with the remaining ingredients.

Stocks of *Chlamydomonas reinhardtii* wild-type strain (WT12) were stored at room temperature in TAP medium. TAP medium contained per liter: TRIS, 2.42 g, TAP salt stock, 25 mL; phosphate solution, 0.375 mL, six Kropat's trace element stock solutions, 1 mL each and glacial acetic acid, 1 mL. The medium was autoclaved for 20 minutes at 121 C. TAP salt stock solution contained (in gL^{-1}): NH_4Cl, 15.0; $MgSO_4 \cdot 7\ H_2O$, 4.0; $CaCl_2 \cdot 2\ H_2O$, 2.0. The phosphate solution contained 28.8 g K_2HPO_4 and 14.4 g KH_2PO_4 and water was added to a volume of 100 mL. Kropat's trace element stock solutions contained: 1) 25 mM EDTA-Na_2; 2) 28.5 mM $(NH_4)_6MO_7O_{24}$; 3) 2 mM $CuCl_2 \cdot 2\ H_2O$ and 2 mM EDTA; 4) 2.5 mM $ZnSO_4 \cdot 7\ H_2O$ and 2.75 mM EDTA; 5) 6 mM $MnCl_2 \cdot 4\ H_2O$ and 6 mM EDTA; 6) 20 mM $FeCl_3 \cdot 6\ H_2O$, 22 mM EDTA and 22 mM Na_2CO_3. For liquid precultures of *C. reinhardtii* Erlenmeyer flasks were filled with fresh TAP medium and inoculated with 5 % (v/v) stock solution and incubated at 25 °C with an 18 hours light to 6 hours dark cycle with 120 $molm^{-2}s^{-1}$ for five days under shaking at 120 rpm.

Membrane bioreactor

The membrane bioreactors were based on Multifors 2 and Labfors 5 bioreactors (Infors HT, Bottmingen/Basel, Switzerland) with 0.5 and 2.7 liter working volume, respectively. Both reactors were modified with a tubular polydimethylsiloxane (PDMS) membrane. The membrane area to volume ratio was 0.3 cm^2mL^{-1} at 0.5 liter scale. Mono-Lumen Tubing 0.64x1.19x0.28 mm (Dow Corning, USA) gave a k_La value with standard error of 3.24±0.05 h^{-1} in the Multifors reactors. The membrane area to volume ratio was 0.3 cm^2mL^{-1} in the Labfors. Mono-Lumen Tubing 1.58x3.18x0.80 mm (Dow Corning, USA) membrane gave a k_La value with standard error of 0.34±0.01 h^{-1}. The membrane was continuously flushed with air at 140 $mLmin^{-1}$ per liter liquid phase. The temperature was set to 30 °C and the pH to 6.0 using 4 N phosphoric acid and 4 M sodium hydroxide. The redox potential and the pH were measured using EasyFerm Plus ORP Arc and EasyFerm Plus PHI Arc probes (Hamilton, Bonaduz, Switzerland). Liquid samples were collected using a UV lamp as the contamination barrier. No external cellulolytic enzyme mixture or nitrogen purging were used. The reactor was autoclaved for 20 minutes at 121 °C with microcrystalline cellulose or pretreated beechwood. The remaining medium components were added when the reactor was inoculated with 5 % (v/v) *T. reesei*. After 48 hours, the lactic acid bacteria were inoculated to an optical density (OD_{600}) of 0.5 by centrifuging the cells at 3000 rpm for 10 minutes and suspending them in fresh Mandels medium. This time was set as time zero. Another 48 hours after inoculation of the LAB, the *C. tyrobutyricum* or *V. criceti* was inoculated to 5 % (v/v) from a two-day old pre-culture. For fed-batch experiments, the feed solution was sterile filtered and fed at a constant feeding rate.

Experiments with the green alga *Chlamydonomas reinhardtii* (WT12) were performed in a custom made 1 L glass stirred tank reactor equipped with the same membrane as in the Labfors fermenters described above and a membrane area to volume ratio of 0.3 cm^2mL^{-1}. In order to enable mixotrophic growth of the microalgae a radially emitting light fiber (side glow fiber, 1.5 mm diameter, Starscape, Northumberland, United Kingdom) connected to an external light source (Cree XLAMP XHP 70.2 – 3000 K 80 CRI) was mounted next to the membrane (**Fig. S14 - S15**). The reactor was autoclaved for 20 minutes at 121 °C with water and microcrystalline cellulose. The remaining medium components were added when the reactor was inoculated at the same time with 5 % (v/v) *T. reesei* and 5 % (v/v) *C. reinhardtii.* For co-culture experiments Mandel's medium was used and the temperature was set to 26 °C.

Steam pretreatment and beechwood analysis

Beechwood chips (*Fagua sylvatica*) from a local forest in Bern, Switzerland, were air-dried (dry matter 94 %) and milled to <1.5 mm. The raw biomass was composed of (% (w/w)) glucan 39.95±1.25, xylan 19.0±0.5, acetic acid 7.3±0.2, acid-insoluble lignin 25.6±0.7, acid-soluble lignin 5.3±0.1, ash 0.5 and extractives 0.9. A two-stage steam pretreatment was applied with a custom-built steam gun (Industrieanlagen Planungsgesellschaft m.b.H., Austria) to target the partial recovery of soluble xylooligosaccharides from hemicellulose in the prehydrolyzate and simultaneously maximize the glucose yield in the enzymatic hydrolysis of the solids. Beechwood was heated to 180 °C by the injection of saturated steam and pretreated for a residence time of 24.8 minutes (severity of 3.75). At constant pressure and temperature, the liquid phase was removed through a circular nozzle located slightly above the lower ball valve. Subsequently, the pressure was slowly released to 2.0 bar, which is below the known pressure needed to see an effect of the explosion (*45*). At 2.0 bar, the lower ball valve opened, and the solids were removed from the reactor. The solids composed of (% (w/w)) 49.0±0.62 glucan, 7.55±0.1 xylan, 4.2±0.1 acetic acid, 29.9±0.73 acid-insoluble lignin and 3.68±0.37 acid-soluble lignin. The recovered solids were treated a second time at 230 °C for 14.1 minutes and the pressure was abruptly released. The solids contained (in % (w/w)) 55.0±1.4 glucan, 2.1±0.1

xylan, 1.2±0.2 acetic acid, 35.4±0.8 acid-insoluble lignin and 2.5±0.1 acid-insoluble. The 180 °C liquid fraction contained (in gL^{-1}): acetic acid, 2.13; formic acid, 0.4; xylose, 1.1; xylooligosaccharides calculated as xylan, 22.35. During acid-hydrolysis, an additional 5.57 gL^{-1} acetic acid was released from deacetylation of xylooligomers. The severity was calculated according to Overend et al. (*46*). Mass fluxes of glucan, xylan, acetic acid and lignin are shown in **Fig. 4A** and further details of recovery percentages can be found in (**Fig. S9**).

Enzyme assays

EG, BG, CBH and xylanase activity assays were performed at pH 5.0 in the presence of 50 mM citrate buffer, 0.02 (w/v) % sodium azide at 50 °C in duplicates. The enzymatic activity was expressed in International units (IU). One Unit is defined as the amount of enzyme that catalyzes the conversion of one micro-mole of substrate per minute. EG, BG and CBH assays were performed according as described recently (*47*)**.** Ultra-low viscosity carboxymethylcellulose (2 (w/w) %) was used as substrate for EG assays, Avicel (2 (w/w) %) for CBH and 4-Nitrophenyl α-D-glucopyranoside (1 mM) for BG. Birch wood xylan (1 (w/w) %) was used as substrate for Xylanase activity measurements (12.5 µL sample in 125 µL total reaction volume). Reducing sugars were quantified using the 3,5-Dinitrosalicylic acid (DNS) method (*48*).

Analytical methods

Acetic acid, lactic acid, propionic acid, butyric acid, formic acid, glucose, cellobiose, xylose, hydroxymethylfurfural and furfural were quantified by high performance liquid chromatography (Waters 2695 Separation Module, Waters Corporation, Milford, MA, USA), using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C with 5 mM H_2SO_4 as the mobile phase a flow at 0.6 $mLmin^{-1}$, a refractive index detector (Waters 410) at 40 °C and a photo diode array detector (Waters 2998) at 210 nm. The detection limit was 0.05 gL^{-1}. Structural carbohydrates and lignin were measured according to standard methods (*49*).

Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids in biofilms of *T. reesei* and *C. reinhardtii* were quantified by first extracting pigments with dimethylformamide (DMF) and measuring the absorbance at 480, 647 and 664.5 nm (Thermo Spectronic UV-1 (Thermo Fisher Scientific, Waltham, USA)). Prior to the measurement samples with a specific volume or mass were centrifuged in a 1.5 mL Eppendorf vial, 1 mL DMF was added to the pellet and the vials were agitated using a vortex at room temperature for 15 minutes. The vials were centrifuged at 10.000 rpm on a benchtop centrifuge. The chlorophyll a, chlorophyll b and total chlorophyll contents were calculated using the equations from Inskeep and Bloom (*50*) and the amount of carotenoids using the equation from Wellburn (*51*).

Autofluorescence from photosynthetic pigments of the stained biofilm samples was also measured using a confocal laser scanning microscope (Leica SP8, Leica Microsystems, Germany). The setup was equipped with a white light laser set to 30 % laser power to excite the sample. The scan speed was 400 Hz and the objective HC PL APO CS2 40x/1.30 oil with a magnification of 40 was installed. Autofluorescence from photosynthetic pigments was detected in the red channel (excitation at 650 nm and emission at 680 to 700 nm). Fluorescein diacetate was used as viability stain. Fluorescein was detected in the green channel (excitation 495 nm and emission at 514 to 521 nm). The fluorescence projections of the biofilm were generated using the Leica LAS software.

Ternary plot of theoretical butyric acid yield for different fractions of glucan, xylan and acetic acid

L. pentosus assimilate hexose through the Embden-Meyerhof-Parnas pathway and xylose through the PK pathway to lactic and acetic acid. *C. tyrobutyricum* forms 2 moles of butyric acid using 3 moles of lactic acid and 1 mol of acetic acid. *C. tyrobutyricum* can convert sugars directly to butyric acid with a theoretical yield of 0.486 gg^{-1} for glucose and 0.493 gg^{-1} for xylose. The glucan to xylan ratio of pretreated beech wood was 2.82 resulting in a maximal yield of 0.488 gg^{-1}. The application of the lactate platform concept increased the theoretical yield by 7.5 % to 0.525 gg^{-1} fermentable carbohydrates. The maximum yield of 0.635 gg^{-1} was found for a glucan/xylan/acetic acid ratio of 81/0/19, which corresponds to an increase of 31 %. When not only the carbohydrates, but also acetic acid is counted as carbon source for the lactate platform, the theoretical yield of the latter case is 0.52 gg^{-1} and 107 % of the theoretical yield for direct sugar conversion (**Fig. 2E**).

Is *T. reesei* metabolically active throughout the fermentation?

While the focus of this work was to examine the synthetic abilities of the different artificial consortia, it is also of interest to determine how the different members are interacting at the different stages and if the consortia function in unison or in a sequential manner. Specifically, we would like to determine, whether *T. reesei* is active also in phase II and III of the lactate platform fermentations.

Hints on the metabolic activity of *T. reesei* can be obtained by measuring the hydrolytic enzyme expression over time and by recording the redox potential. Based on the batches run on pretreated beechwood, we found for each of the three consortia increasing activities of cellobiohydrolases (CBH), endoglucanases (EG), beta-glucosidases and xylanases in the supernatants also after inoculation with the product forming strain (**Fig. S11**). As an additional control, we compared the lactic acid production profiles on microcrystalline cellulose where in one reactor the aeration and with that the metabolic activity of *T. reesei* was stopped at the time of inoculation with *L. pentosus*. The lactic acid yield after 275 h in the reactor with continued aeration was 35 % higher than in the reactor without aeration **(Fig. S18**). This can be attributed to a higher enzyme activity in the aerated reactor and thus, it indicates that *T. reesei* stays metabolically active and continues to produce enzymes in the presence of *L. pentosus*. This also confirms previous results obtained for CBP to ethanol and lactic acid, where enzyme production in semi-continuous experiments was observed (*25*, *26*). However, a substantial amount of enzyme is produced in the first phase, where *T. reesei* is forming biomass to build the biofilm. This behavior was also observed on axenic cultures of the fungus (*47*).

Another indication of the metabolic activity of *T. reesei* can be drawn by measuring the dissolved oxygen concentration (DO) in a consortium, where the fungus is the only species that can consume oxygen. This is the case when a consortium of *T. reesei* and a respiration deficient yeast is used as fermenting strain. In such a co-culture, the DO value was zero for 112 h thereby proving the metabolic activity of *T. reesei*. When sodium azide was added to kill the fungus, the DO signal increased immediately (**Fig. S19**). In all presented lactate platform fermentations, DO was below the detection limit of the sensor and the redox potential was always lower than -150 mV. Here however, both *L. pentosus* and *T. reesei* could be responsible for oxygen uptake and the redox potential is clear indication, that at least one of these two species was active until the end of the experiments. *L. pentosus* does not have a respiratory chain but consumes 1 mol of oxygen for heterofermentative conversion of 1 mol glucose. With this stoichiometry and the characteristic oxygen transfer capacity of the membrane reactor, we estimated the minimal glucose consumption rate of *L. pentosus* that is necessary to maintain anaerobic conditions in the reactor. With a measured k_La of 0.34±0.01 h^{-1} and an assumed maximal dissolved oxygen concentration of 6.5 mgL^{-1}, the maximal oxygen transfer rate of the membrane reactor is

21 $mgL^{-1}h^{-1}$. Thus, the heterofermentative glucose consumption rate has to exceed a value 118 $mgL^{-1}h^{-1}$ to maintain a DO of 0 %. On the other hand, sole respiration of *T. reesei* requires 6 moles of oxygen for 1 mol of glucose and for this reaction, a glucose conversion rate of only 20 $mgL^{-1}h^{-1}$ or higher is sufficient to maintain anaerobic conditions. A linear glucose consumption rate of 118 $mgL^{-1}h^{-1}$ translates in the complete conversion of 17.5gL^{-1} microcrystalline cellulose within 165 h. However, actual fermentation times were much longer, and the limiting cellulose hydrolysis rate is not constant, but high in the beginning and much lower at the end. Furthermore, *L. pentosus* is known to be primarily a homofermentative strain and it is unlikely that only heterofermentative glucose consumption takes place as was assumed in the calculation above. In order to test the last hypothesis, we performed an axenic fed batch fermentation of *L. pentosus* with a glucose feeding rate of 100 and 200 $mgL^{-1}h^{-1}$. In both cases, aerobic conditions were maintained, and the dissolved oxygen concentrations were above 58% throughout the whole experiment (**Fig. S20**).

Taken together, we conclude that it is highly unlikely that solely *L. pentosus* is able to maintain anaerobic conditions in the reactor. Consequently, *T. reesei* stayed metabolically active and consumed oxygen throughout the whole duration of the presented experiments.

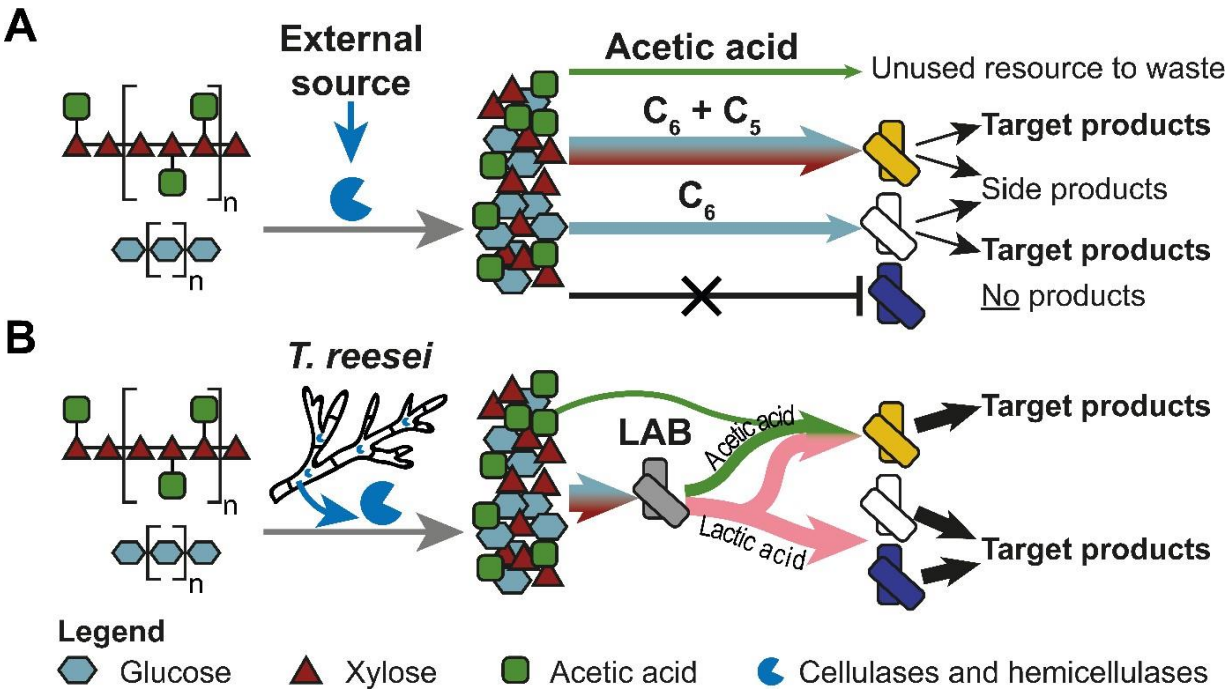

Fig. S1. Comparison of the sugar platform and the lactate platform. (A) Schematic illustration of the common sugar Platform. The polymeric compounds glucan and xylan are hydrolyzed by cellulases and hemicellulases added from an external source to yield a heterogeneous mixture of glucose and xylose together with acetic acid which is released from the xylopuranose backbone of xylan. Many natural product-forming microorganisms lack the metabolic flexibility to efficiently and selectively convert this heterogeneous carbohydrate mixture to the target products, and usually acetic acid is not utilized as carbon source. Increased by-product formation and reduced product yield and concentration are the major challenges. **(B)** Schematic illustration of the lactate platform. *T. reesei* is introduced as *in situ* producer of cellulolytic enzymes. The heterogeneous mixture of carbohydrates is metabolically funneled to lactic acid, which is further converted to the target products. This reduces the metabolic flexibility required of the target product-forming microorganisms. Furthermore, some product forming strains are able to co-consume lactic and acetic acid which results in lower by-product formation.

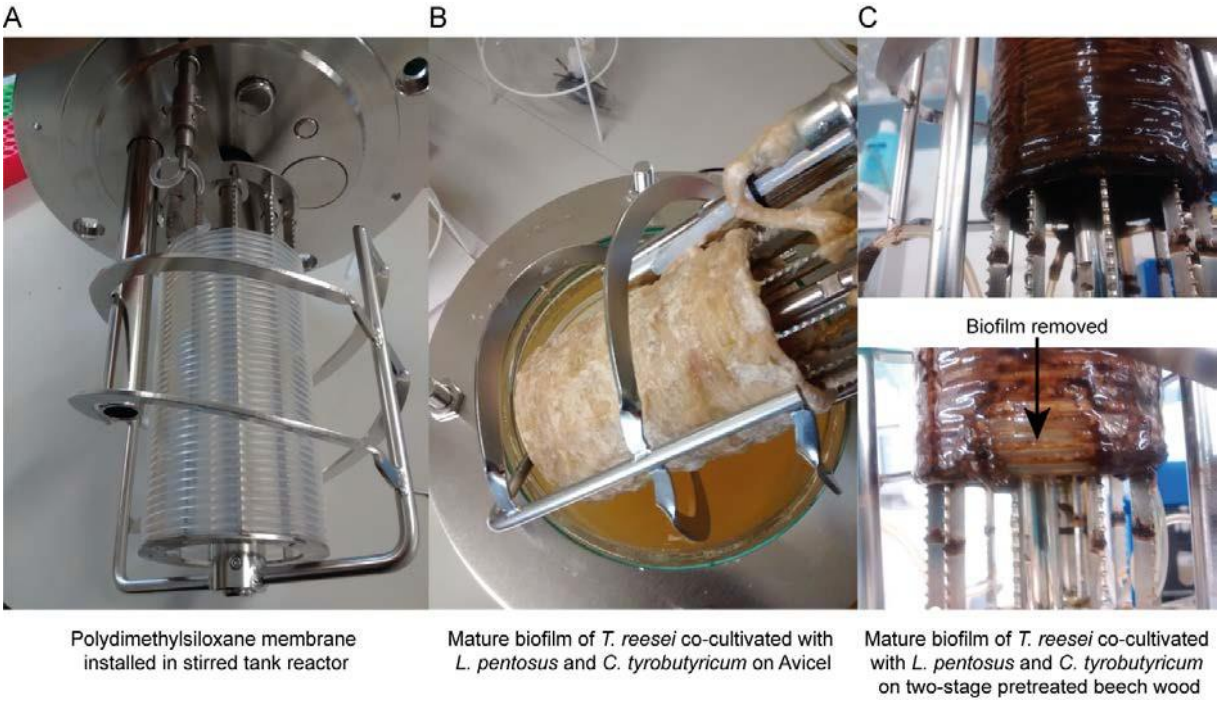

Fig. S2. Pictures of the membrane installed in stirred tank bioreactors. (A) Without biofilm, **(B)** with a mature fungal biofilm grown on Avicel and **(C)** with a mature fungal biofilm grown on pretreated beechwood**.**

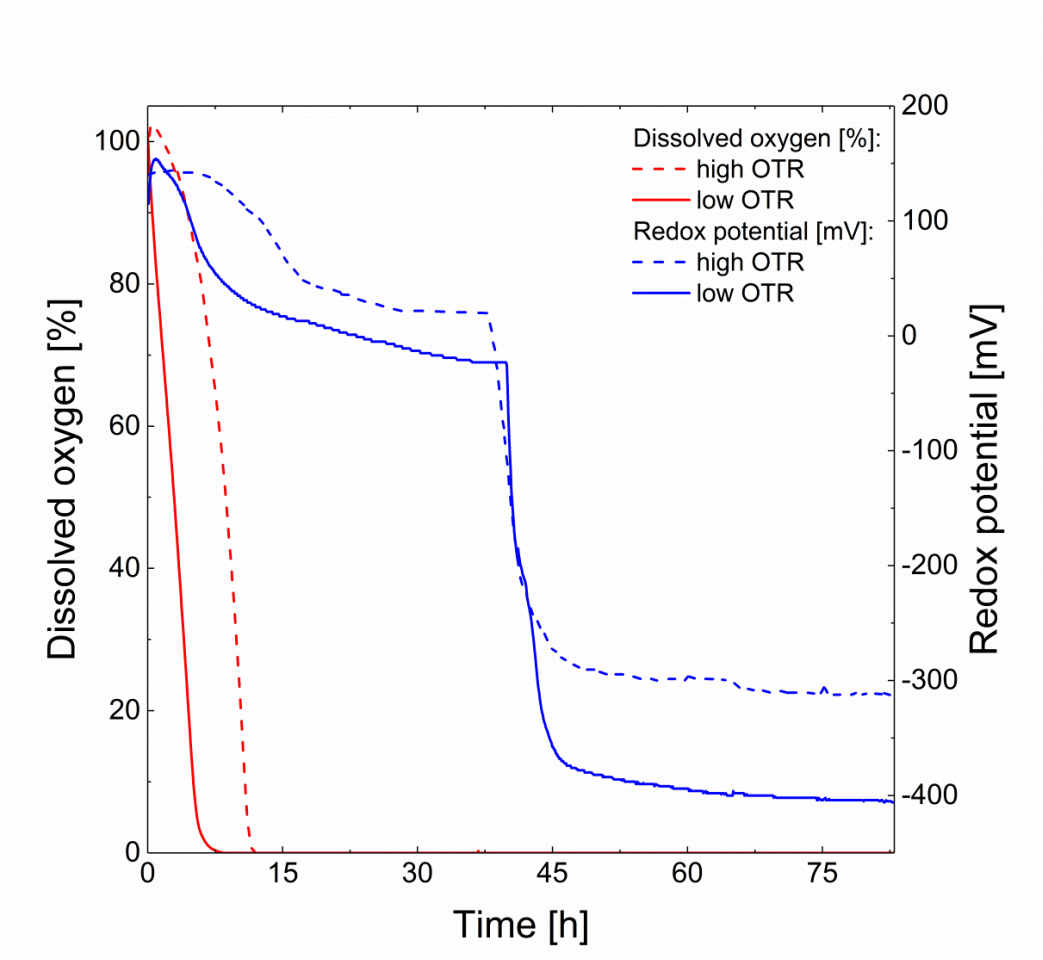

Fig. S3. Redox potential and dissolved oxygen concentration in the two-stage inoculation procedure using the membrane bioreactor. *T. reesei* was inoculated at time zero. The dissolved oxygen concentration dropped below the detection limit after eight hours in the low oxygen transfer rate (OTR) approach (0.34 h^{-1}) and after 12 hours in the high OTR approach (3.24 h^{-1}). Due to the metabolic activity of *T. reesei*, the redox potential was lowered to approximately +40 mV (high OTR) and -20 mV (low OTR). After approximately 40 hours, *L. pentosus* was inoculated and the redox potential dropped below -300 mV (high OTR) and -400 mV (low OTR) approach.

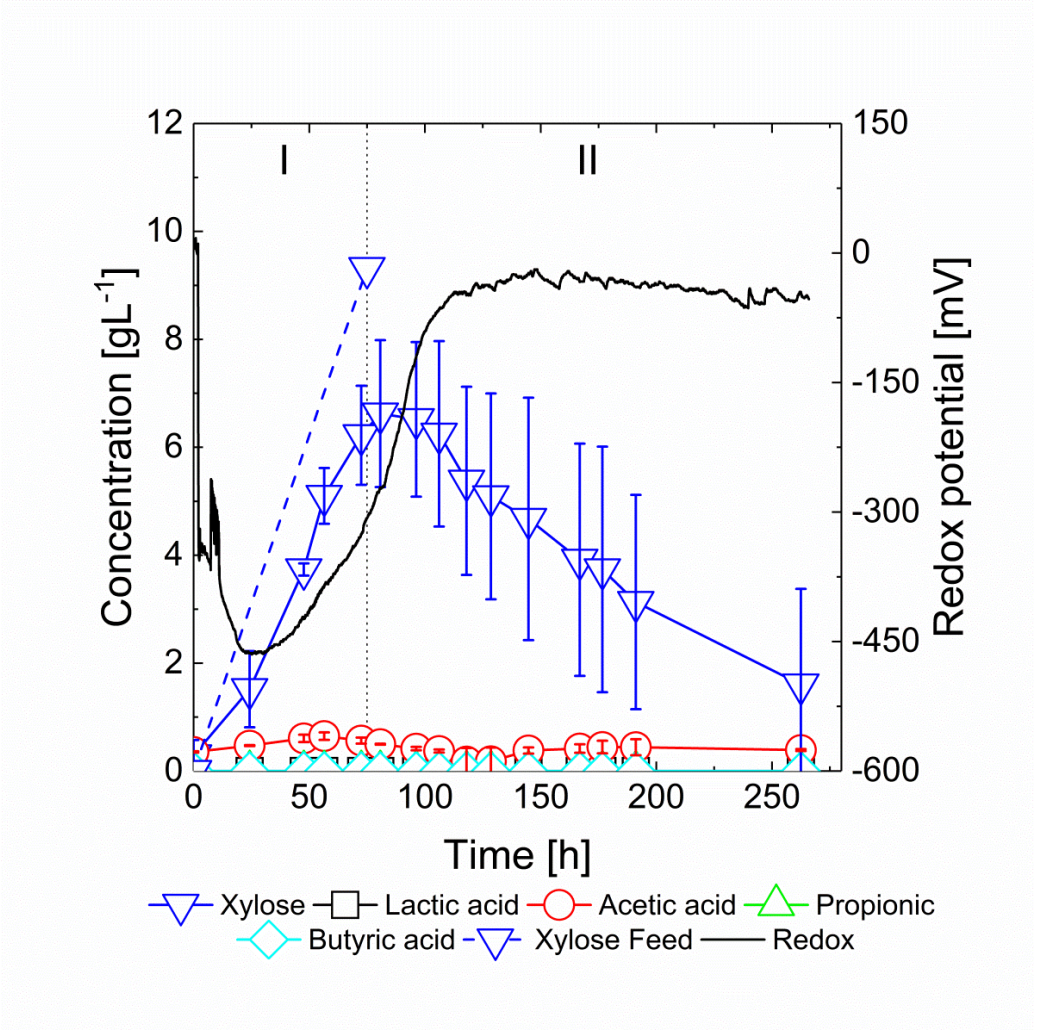

Fig. S4. CBP for direct production of butyric acid from microcrystalline cellulose and xylose with a co-culture of *T. reesei* and *C. tyrobutyricum*. In the high OTR approach (3.24 h^{-1}), *C. tyrobutyricum* was inoculated at time zero, 48 hours after *T. reesei*. In phase I, a linear xylose feed was applied to an accumulated concentration of 9.32 gL^{-1}. The redox potential dropped temporarily due to the presence of reducing agents from the pre-culture of *C. tyrobutyricum*, but increased rapidly to a level unsuitable for the cultivation of the obligate anaerobe *C. tyrobutyricum*, which therefore exhibited only temporary metabolic activity.

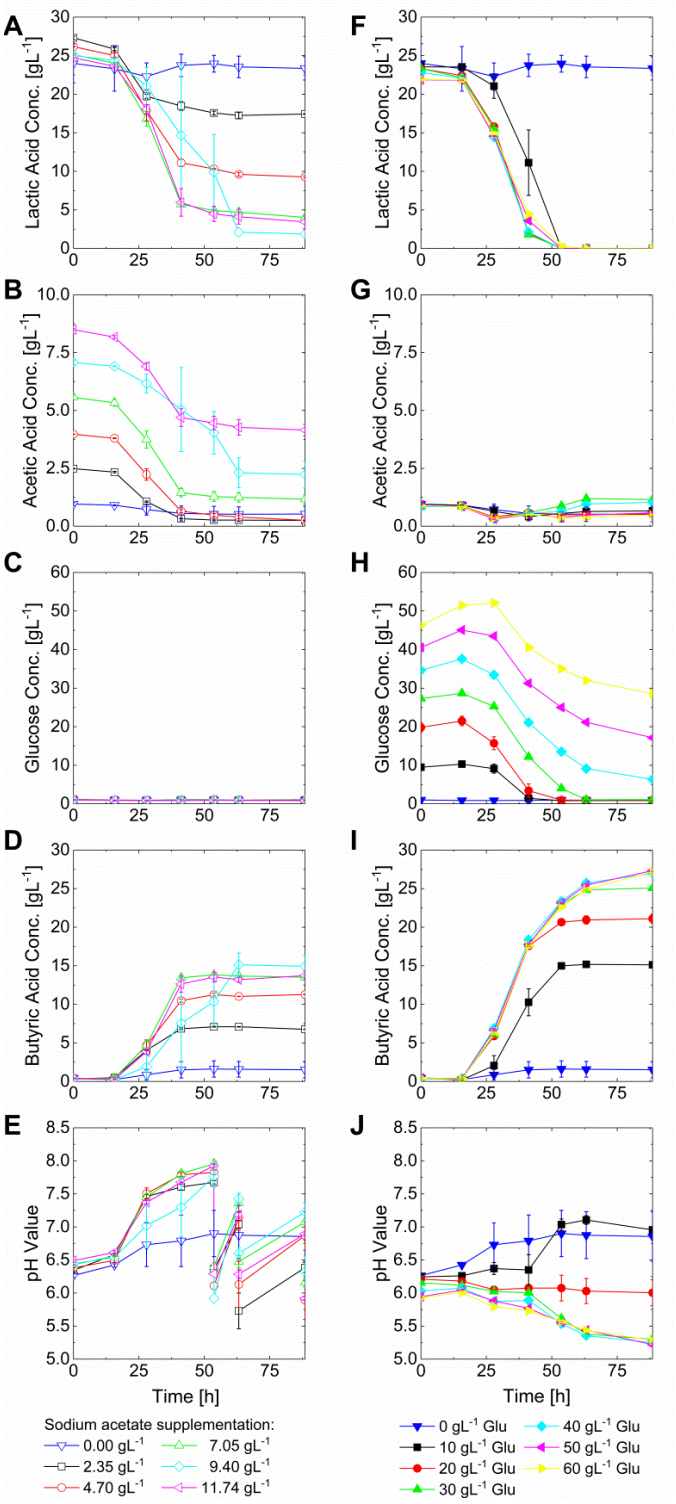

Fig. S5. Characteristics of *C. tyrobutyricum* grown on lactate broth supplemented with sodium acetate (A-E) or glucose (F-J). The lactate broth was obtained using the consolidated bioprocess (CBP) with 35 gL^{-1} microcrystalline cellulose and 18.64 gL^{-1} xylose as substrates. *C. tyrobutyricum* is unable to use lactic acid as its sole carbon source and is therefore unable to grow in the lactate broth without supplementation. Since *C. tyrobutyricum* co-utilizes lactic acid and acetic acid, we supplemented the lactate broth with different concentrations of sodium acetate (i) to test if *C. tyrobutyricum* required additional nutritional supplementation and (ii) to determine the required lactic to acetic acid ratio to completely utilize both carbon sources **(A-E)**. The addition of only sodium acetate already enabled its co-utilization with lactate and the production of butyrate **(A-E)**, so no further complex medium supplementation was tested. *C. tyrobutyricum* stopped consuming lactic acid when the acetic acid was exhausted. In order

to determine the required lactic to acetic acid ratio, we calculated the lactic acid consumed by subtracting the final lactic acid concentration from the initial one, and divided by the concentration of acetic acid obtained by subtracting the final acetic acid concentration from the initial one. We determined a lactic to acetic acid ratio of 4.50±0.07 gg^{-1} or 3.01±0.05 $molmol^{-1}$, averaged from experiments shown in **(A-E),** which is similar to literature data (*52*). Due to the re-utilization of lactic and acetic acid and the formation of butyric acid by *C. tyrobutyricum*, the molar concentration of acids was halved. The pH increased and the addition of hydrochloric acid at 53.7 and 63.2 hours was necessary to lower it again.

In order to avoid the external addition of sodium acetate, we studied the ability of *C. tyrobutyricum* to co-utilize lactate and glucose. By adding glucose in increments of 10 gL^{-1}, up to a maximum of 60 gL^{-1} **(F-J)**, we showed that 10 gL^{-1} were required for complete utilization of the lactate without accumulation of acetic acid (0.42 g glucose/g consumed lactic acid). The utilization of lactic acid suggests the ability of *C. tyrobutyricum* to convert glucose to acetic acid with its subsequent co-consumption with lactic acid to butyric acid. Since the most energy efficient electron bifurcation pathway does not result in sufficiently high amounts of acetic acid, we suggest that a self-regulated metabolic shift towards a less energy efficient pathway for production of acetic acid is occurring (**Tab. S1**). While the pH increased slightly with 10 gL^{-1} glucose, it remained constant at 20 gL^{-1}, which indicates a dynamic equilibrium of the molarity of the acids. Higher levels of glucose supplementation resulted in a drop of the pH after the concentration of lactic acid fell below the detection limit. The remaining glucose was mainly converted to butyric acid. The pH was not adjusted externally during the experiments shown in **(F-J)**.

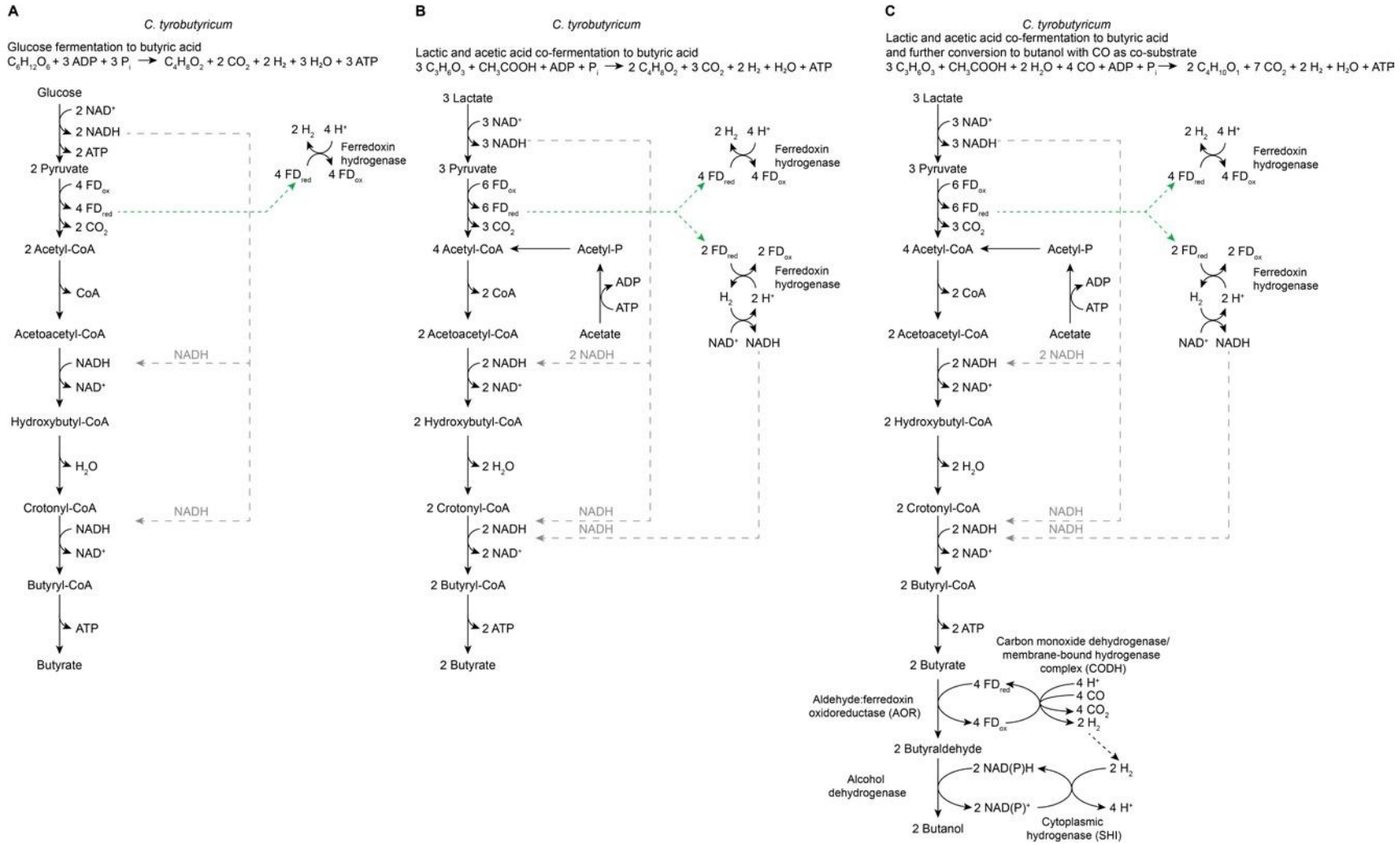

Fig. S6. Selected pathways in *C. tyrobutyricum* for the formation of butyrate or butanol. (A) Glucose fermentation to butyrate. **(B)** Co-fermentation of lactate and acetate to butyrate. In pathway **(A)** and **(B)**, the reduced ferredoxins are reoxidized by hydrogen formation catalyzed by hydrogenases. When lactate/acetate are co-fermented the hydrogen is partially used to regenerate NADH. The energy yield differs such that one mole glucose generates 3 moles ATP, whereby 3 moles lactate and 1 mol acetate generate 1 mol ATP. **(C)** Proposed theoretical pathway for the co-fermentation of lactate and acetate to butanol via butyrate and co-utilization of carbon monoxide. The pathway in **(C)** requires the genetic modification of *C. tyrobutyricum* and the insertion of an aldehyde-ferredoxin oxidoreductase, an alcohol dehydrogenase and a carbon monoxide dehydrogenase/membrane-bound hydrogenase complex which is inspired by Basen et al. (*53*).

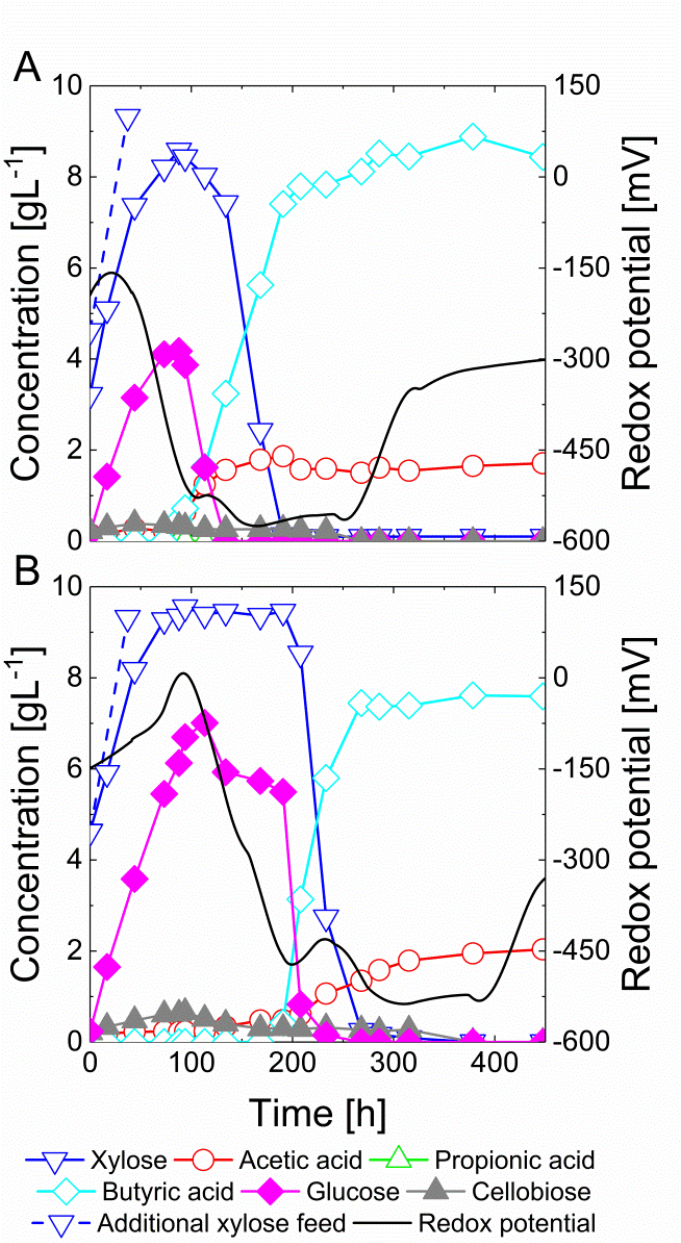

Fig. S7. Simultaneous saccharification and fermentation (SSF) by *C. tyrobutyricum* in a fed-batch experiment. 17.5 gL^{-1} Avicel, 4.7 gL^{-1} xylose and cellulase (15 FPU per gram of cellulose, Accellerase©1500 DuPont, Wilmington, DE) was introduced in one portion. Xylose was fed at a constant rate to an accumulated concentration of 9.32 gL^{-1} in 37.8 hours (dashed blue line). The temperature was set to 30 °C, the pH to 6.0 and the culture was continuously sparged with nitrogen, until *C. tyrobutyricum* showed metabolic activity **(A)** 200 hours, **(B)** 250 hours). The reactor volume was 2.7 L. The final butyric acid to acetic acid ratio was 4.33±0.60. **(A)** and **(B)** represent two duplicates of two independent experiments.

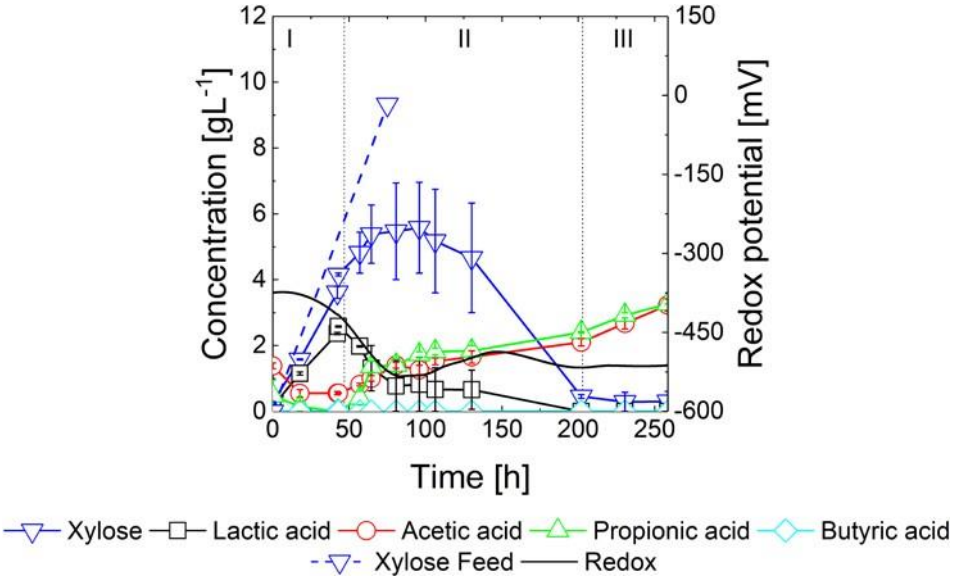

Fig. S8. Fed-batch experiment for CBP to propionic and acetic acid using *T. reesei*, *L. pentosus*, *V. criceti*. 17.5 gL^{-1} Avicel was introduced in one portion and xylose (dashed blue line) was fed at a constant rate to an accumulated concentration of 9.32 gL^{-1} in 75 hours in the low OTR approach (0.34 h^{-1}). Error bars represent the standard deviation from two independent fed-batch experiments.

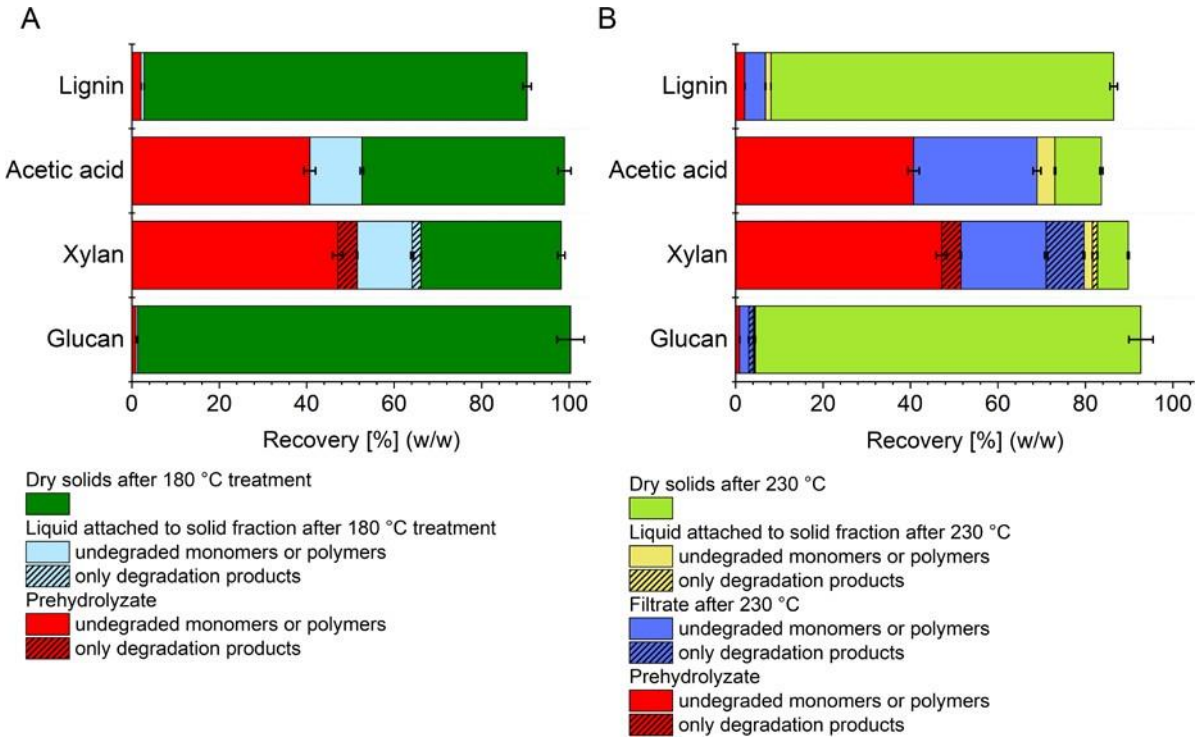

Fig. S9. Steam pretreatment of beechwood: Recovery of lignin, acetic acid, xylan and glucan after (A) the first stage at 180 °C and (B) the second stage at 230 °C. The recovery is quantified as a mass percent of input (raw) biomass. Detected degradation products were expressed as glucan or xylan using the following pathways: formic acid is the degradation product of furfural (hydrolytic fission of the aldehyde group (*54*, *55*)), which itself is the degradation product of xylose. Hydroxymethyl furfural is the degradation product of glucose.

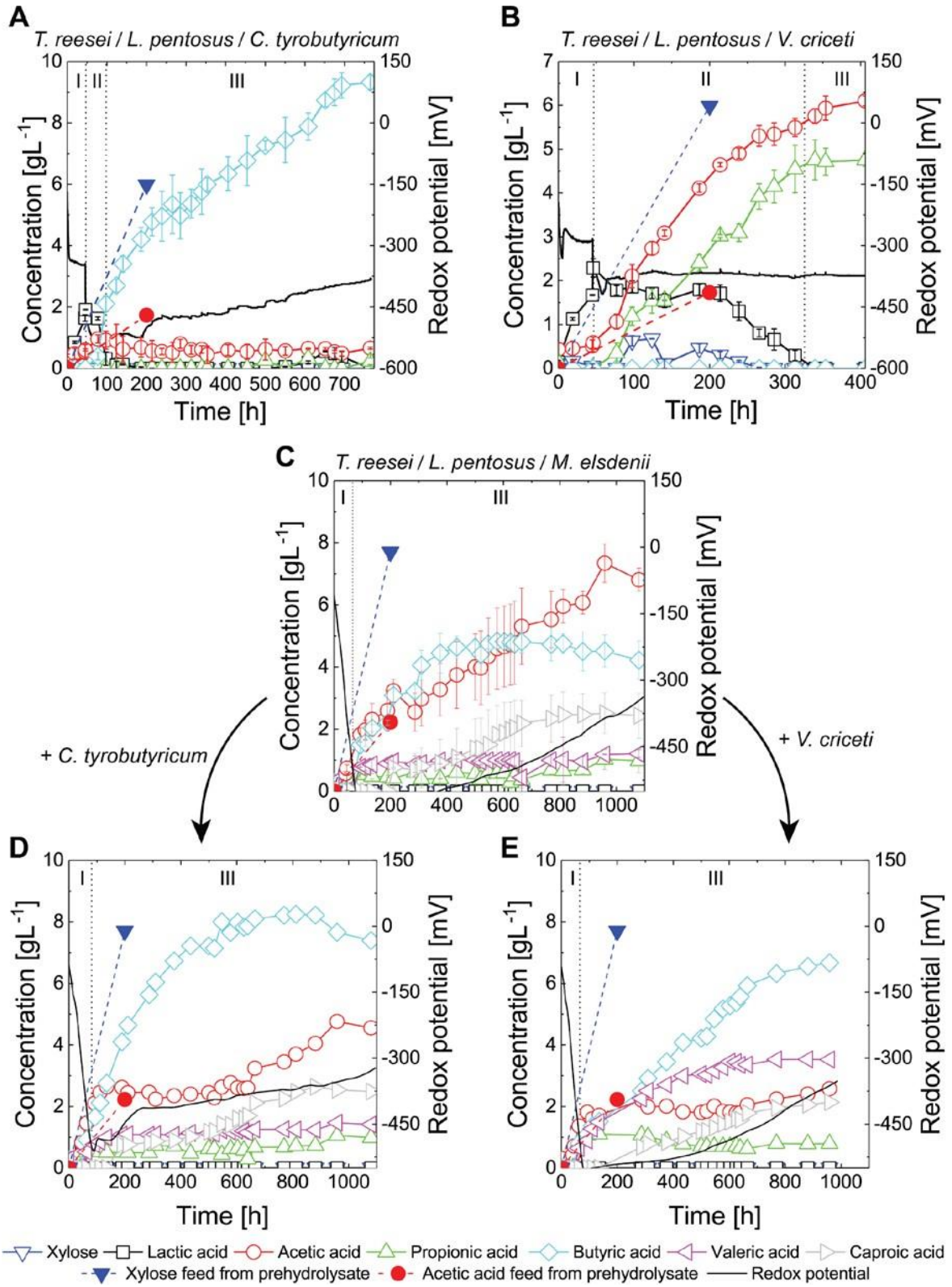

Fig. S10. CBP of pretreated beechwood to SCFAs. CBP of 3 % (w/w) **(A-B)** or 3.86 % (w/w) **(C-E)** two-stage steam-pretreated beechwood solids, respectively, with a feed of the corresponding prehydrolyzate over a period of 200 hours **(A)** to butyric acid using *C. tyrobutyricum*, **(B)** to propionic and acetic acid using *V. criceti* and to mixed VFAs with up to six carbon atoms using **(C)** *M. elsdenii*, **(D)** *M. elsdenii* and *C. tyrobutyricum* and **(E)** *M. elsdenii* and *V. criceti*. The dashed blue line shows the amount of xylose in the form of xylooligomers and the dashed red line the amount of acetic acid added with the prehydrolyzate. A low OTR (0.34 h^{-1}) was applied. Error bars represent the standard deviation from two independent experiments.

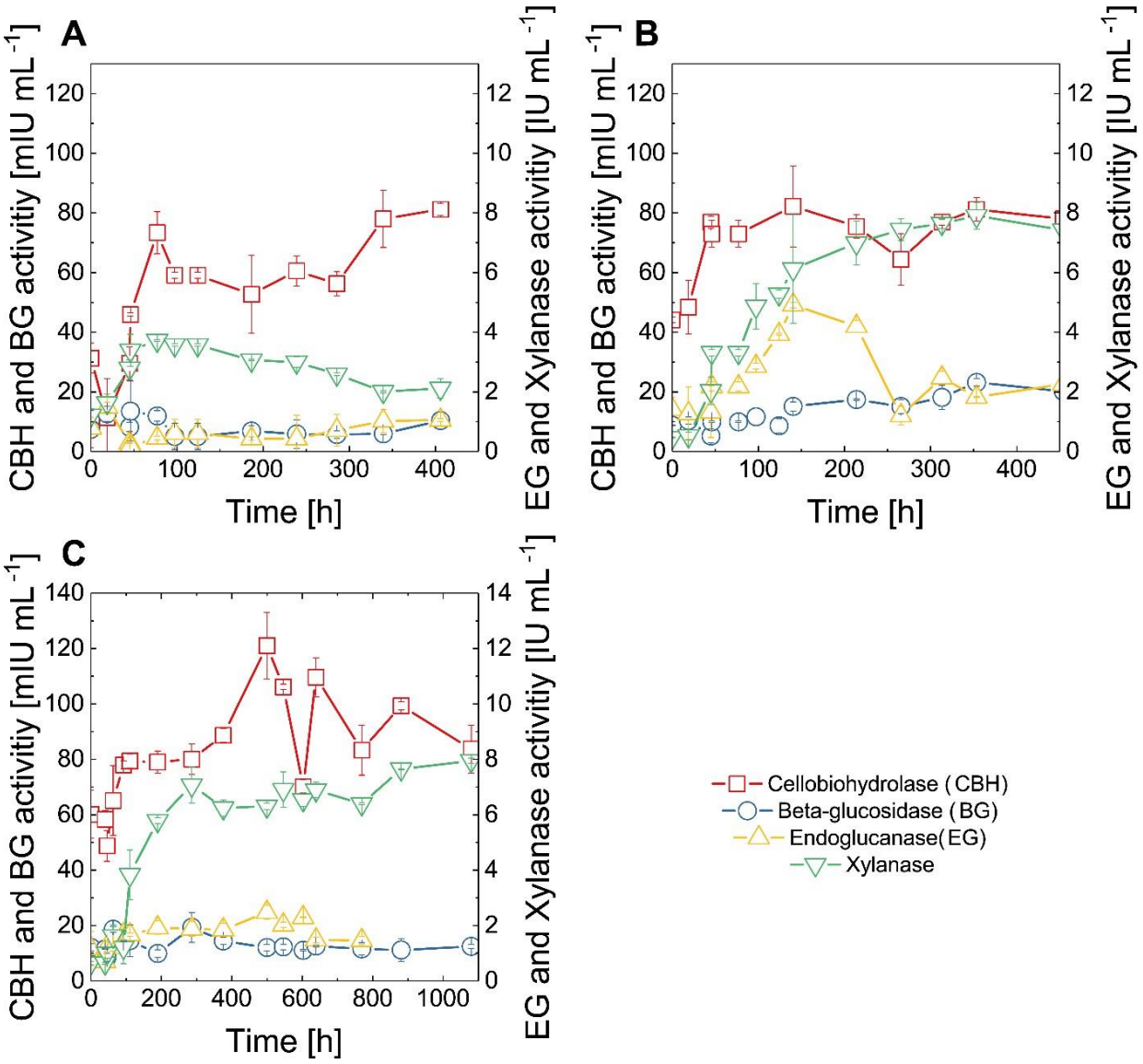

Fig. S11. Activity of cellobiohydrolase (CBH), beta-glucosidase (BG), endoglucanase (EG) and xylanase in the supernatant of the fermentation slurry during various stages of the lactate platform fermentations shown (A) in Fig. S10A, (B) in Fig. S10B and (C) in Fig. S10C. Error bars represent the standard deviation from duplicate assays.

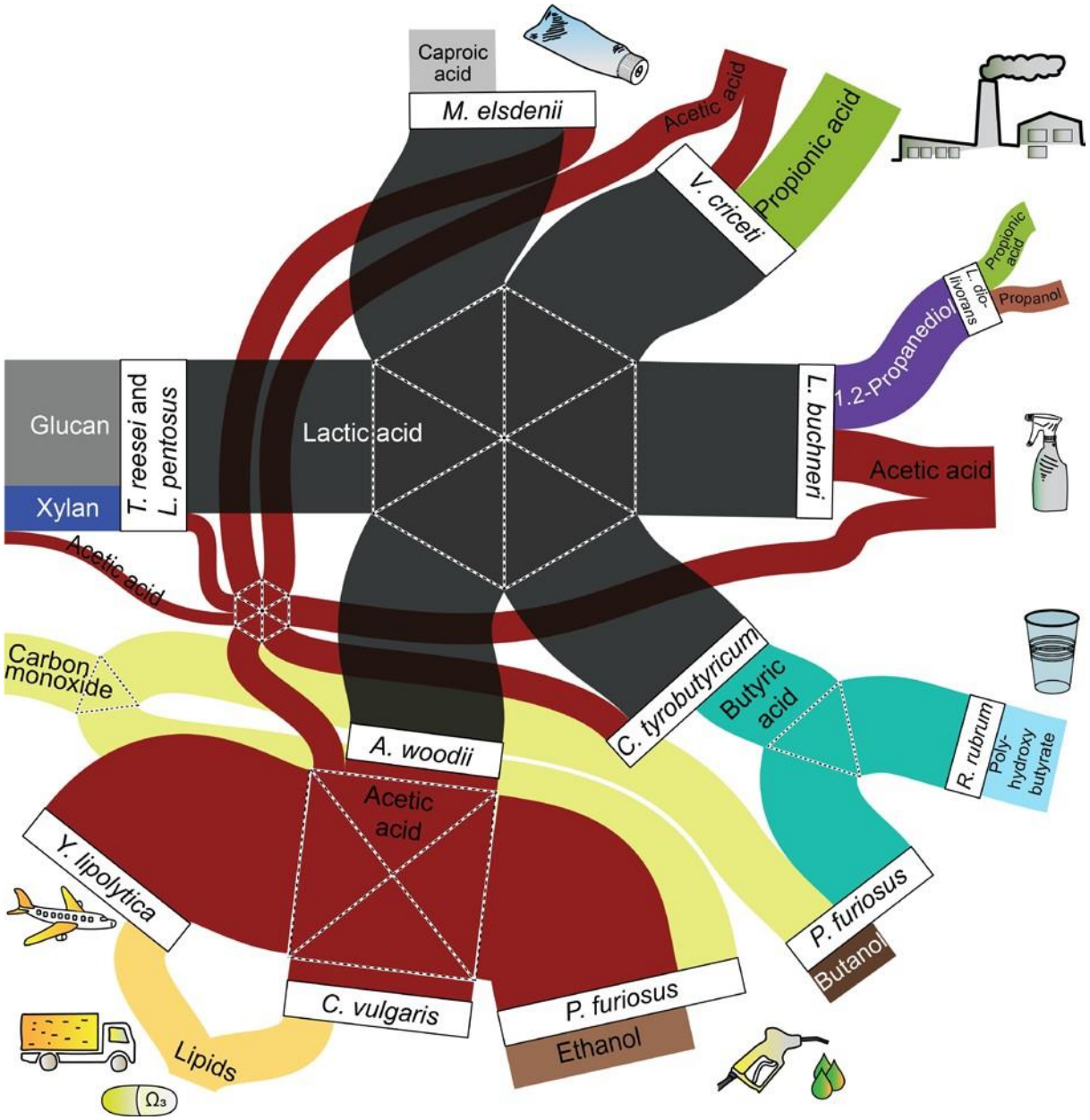

Fig. S12. CBP of pretreated beechwood via the lactate platform to selected target products. Pretreated beech wood composed of glucan, xylan and acetic acid is converted to lactic acid by *T. reesei* and *L. pentosus*. Lactic acid is the central intermediate in further conversions to a variety of products. The thickness of the lines corresponds to calculated theoretical mass fluxes based on pathways shown in **Tab. S8** and **Tab. S9**. Side products such as water, carbon dioxide and hydrogen are not illustrated. We have validated the conversions for lactic acid, acetic acid, propionic acid, butyric acid, valeric acid and caproic acid in the main manuscript. Corresponding theoretical yields for bioconversion of glucose, xylose, acetic acid or two-stage pretreated beechwood (glucose, xylose, acetic acid) via the lactate platform compared to alternative direct fermentations are summarized in **Tab. S7**. The production of alcohols from the corresponding carboxylic acids can also be realized by the genetic modification of *C. tyrobutyricum*. **Fig. S6C** shows the proposed pathway inspired by the experiments performed by Basen et al. (*53*).

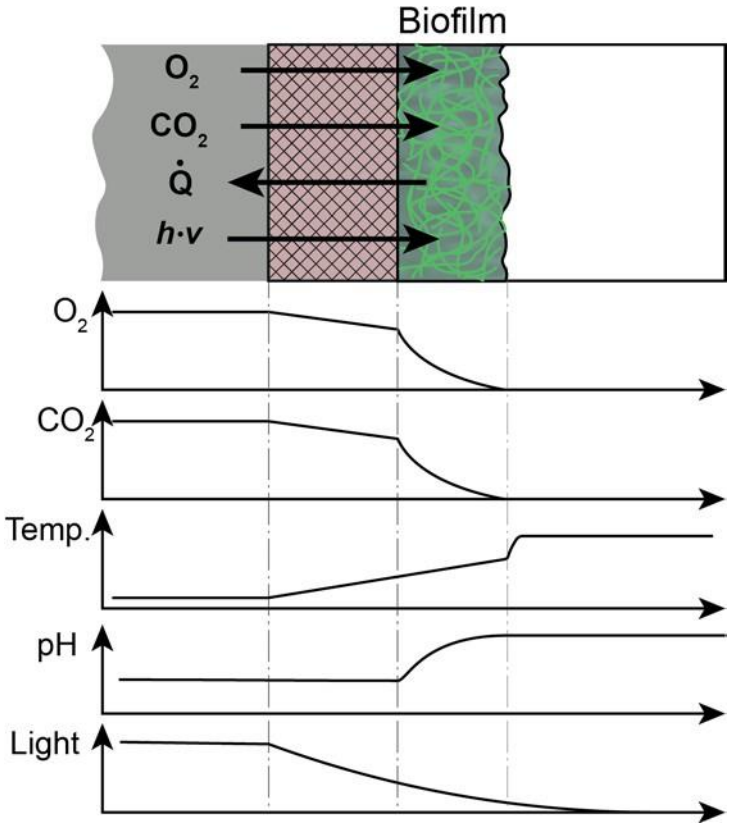

Fig. S13. Reactor cross-sections with different gradients of abiotic factors. Measures to stabilize communities composed of microorganisms that have non-overlapping tolerance ranges by niche differentiation.

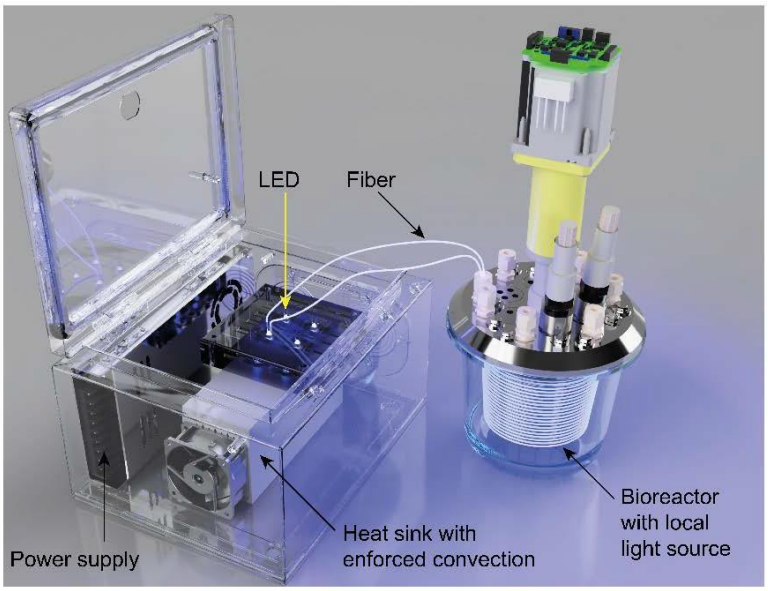

Fig. S14. External light source with radially emitting light fiber connected to the bioreactor. LEDs (Cree XLAMP XHP 70.2 – 3000 K 80 CRI) (*56*) are glued on top of a heat sink with enforced convection. The light is coupled into both ends of a side-emitting light fiber which is helically coiled around the membrane holding structure in the interior of the reactor.

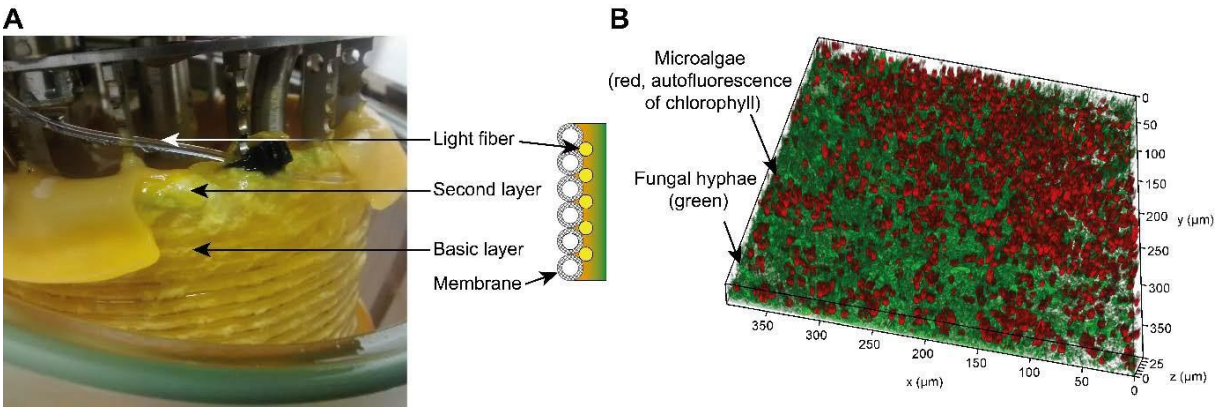

Fig. S15. **Photograph and Confocal Laser Scanning Microscopy Image of Fungal-Microalgal Biofilm Grown on the Surface of the Aerated-Membrane. (A)** Photograph of microalgal-fungal biofilm attached to the membrane which is helically coiled around the membrane-holding structure next to the light fiber. A slightly green second layer in the upper part of the biofilm is on top of a basic layer. **(B)** Confocal laser scanning microscopy (CLSM) z-stack of the biofilm shown in **(A)**. Higher z values indicate an increased distance from the surface of the membrane (source of oxygen). The image was taken about one week after inoculation of both microorganisms.

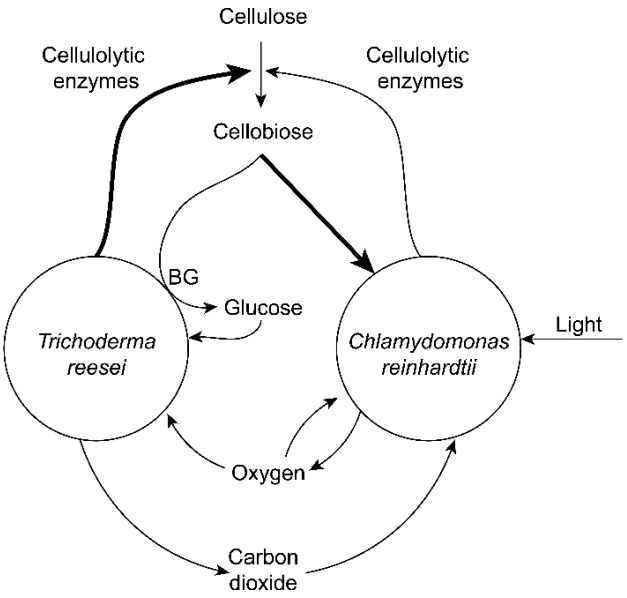

Fig. S16. Overview of pathways in a co-culture of *T. reesei* (fungi) and *C. reinhardtii* (algae) using cellulose as carbon source and light as energy source. The thickness of the arrows indicates increased production of cellulolytic enzymes by *T. reesei* compared to *C. reinhardtii* and increased cellobiose consumption of *C. reinhardtii* compared to *T. reesei*, respectively. Beta-glucosidase (BG).

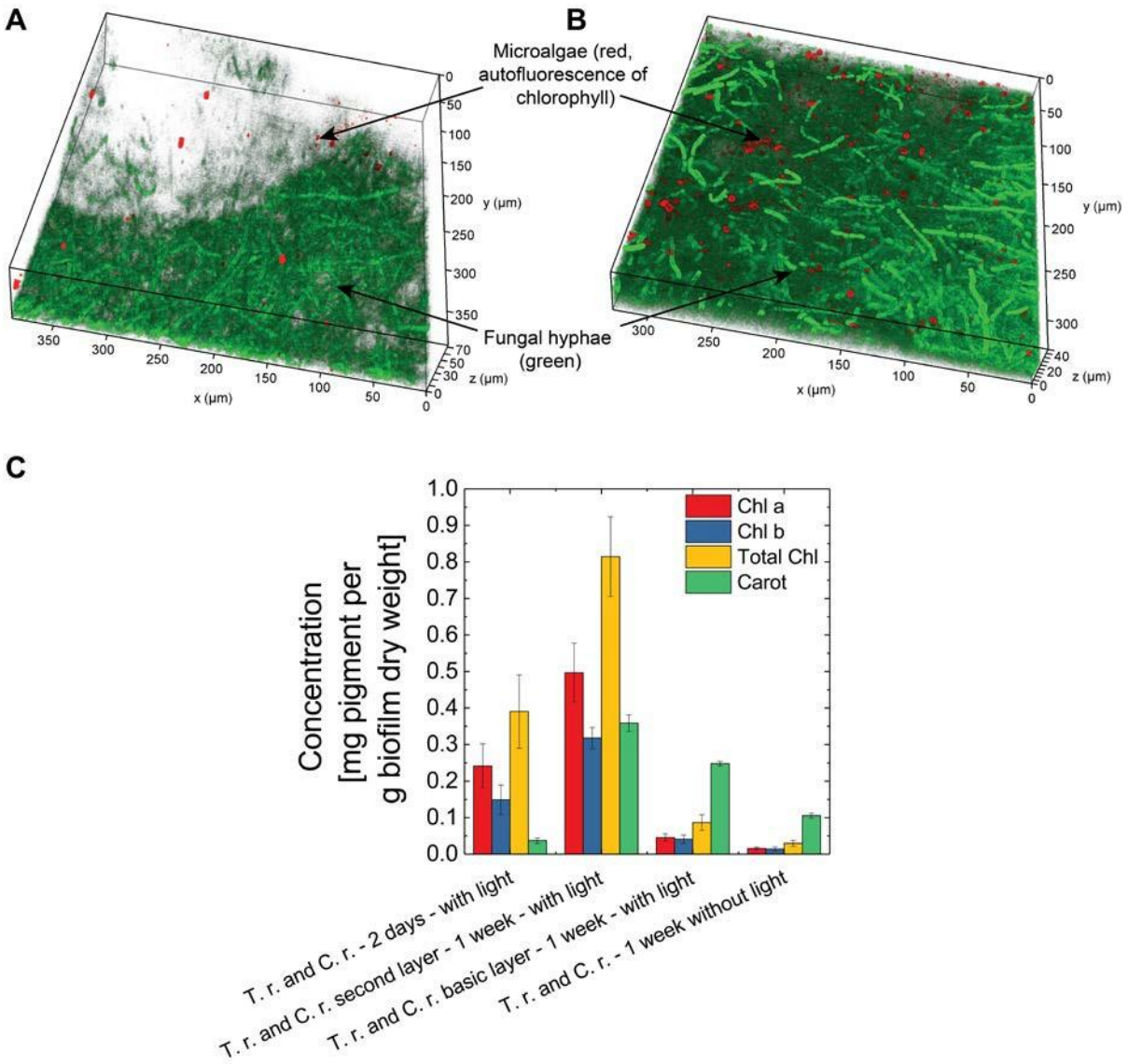

Fig. S17. Confocal laser scanning microscopy images of fungal-microalgal biofilm grown on the surface of the aerated-membrane with and without illumination. (A, B) CLSM z-stack images about five days after inoculation of *T. reesei* and *C. reinhardtii*: **(A)** without illumination, **(B)** with illumination. The biofilm was stained with fluorescein diacetate.
(C) Chlorophyll concentration in biofilm samples from cultivations of *T. reesei* and *C. reinhardtii* with and without light. Pigments from biofilm samples are extracted using the solvent dimethylformamide. A spectrophotometric determination is performed at 480, 647 and 664.5 nm. Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Total Chl) are calculated using the equations from Inskeep and Bloom (*50*). The total amount or carotenoids (Carot) is calculated using the equation from Wellburn (*51*).

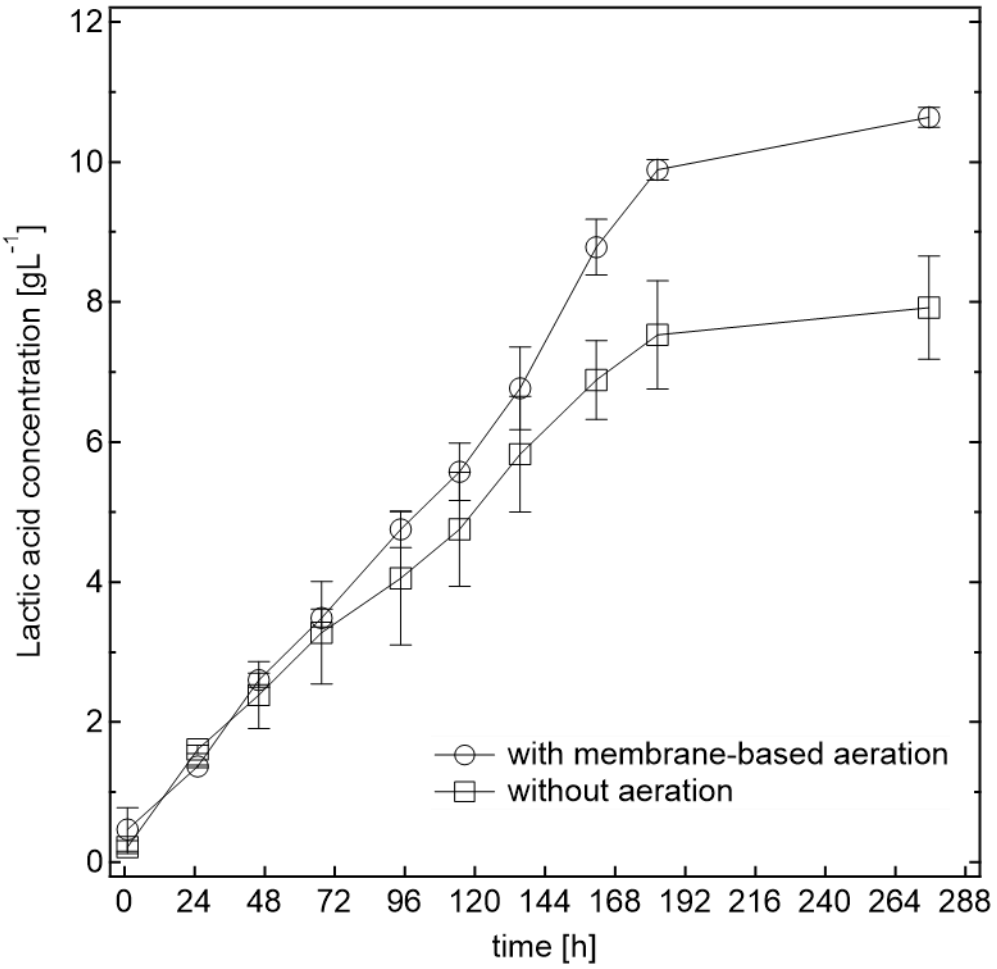

Fig. S18. Influence of the aeration on the production of lactic acid in co-cultures of *T. reesei* and *L. pentosus*. *T. reesei* was grown in duplicates on 17.5 gL^{-1} microcrystalline cellulose for 48 h in membrane aerated biofilm reactors prior to inoculation with *L. pentosus* (designated with t=0). In one reactor the aeration was stopped, while it was continued in the other reactor. The lactic acid yield after 275 h in the reactor with continued aeration was 35 % higher than in the reactor without aeration. This shows that *T. reesei* continued to actively produce cellulolytic enzymes after inoculation with *L. pentosus.*

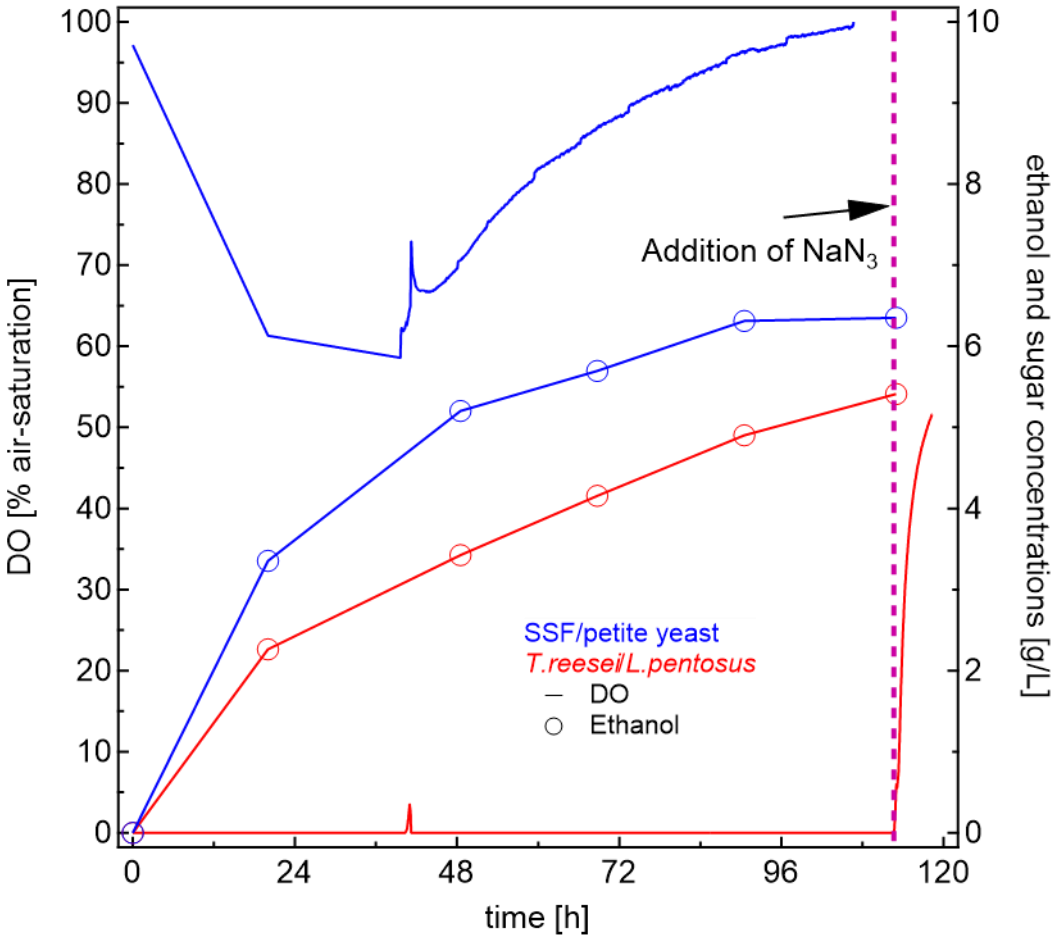

Fig. S19. Comparison of the DO profiles of a co-culture of *T. reesei* and the respiration deficient yeast *S. cerevisiae* FYdelta pet191::KanMX4 and an axenic SSF culture of the same yeast. In the co-culture (shown in red), the DO concentration was zero throughout the whole fermentation thereby proving the metabolic activity of *T. reesei* in the presence of a microbe that consumes glucose but not oxygen. After 112 h, sodium azide was added to a concentration of 1 gL^{-1}, which killed the fungus and resulted in an immediate increase of the DO signal. This control proved that the DO signal is indeed rising, if the biofilm is dead. Shown in blue is the control SSF experiment, where only the petite yeast is growing in a membrane aerated bioreactor. Due to a computer problem, the DO recording started only after 40 h. Prior to that, only two manually recorded values are available. *S. cerevisiae* FYdelta pet191::KanMX4 was generously provided by Professor Stephen G. Oliver, University of Cambridge (*57*).

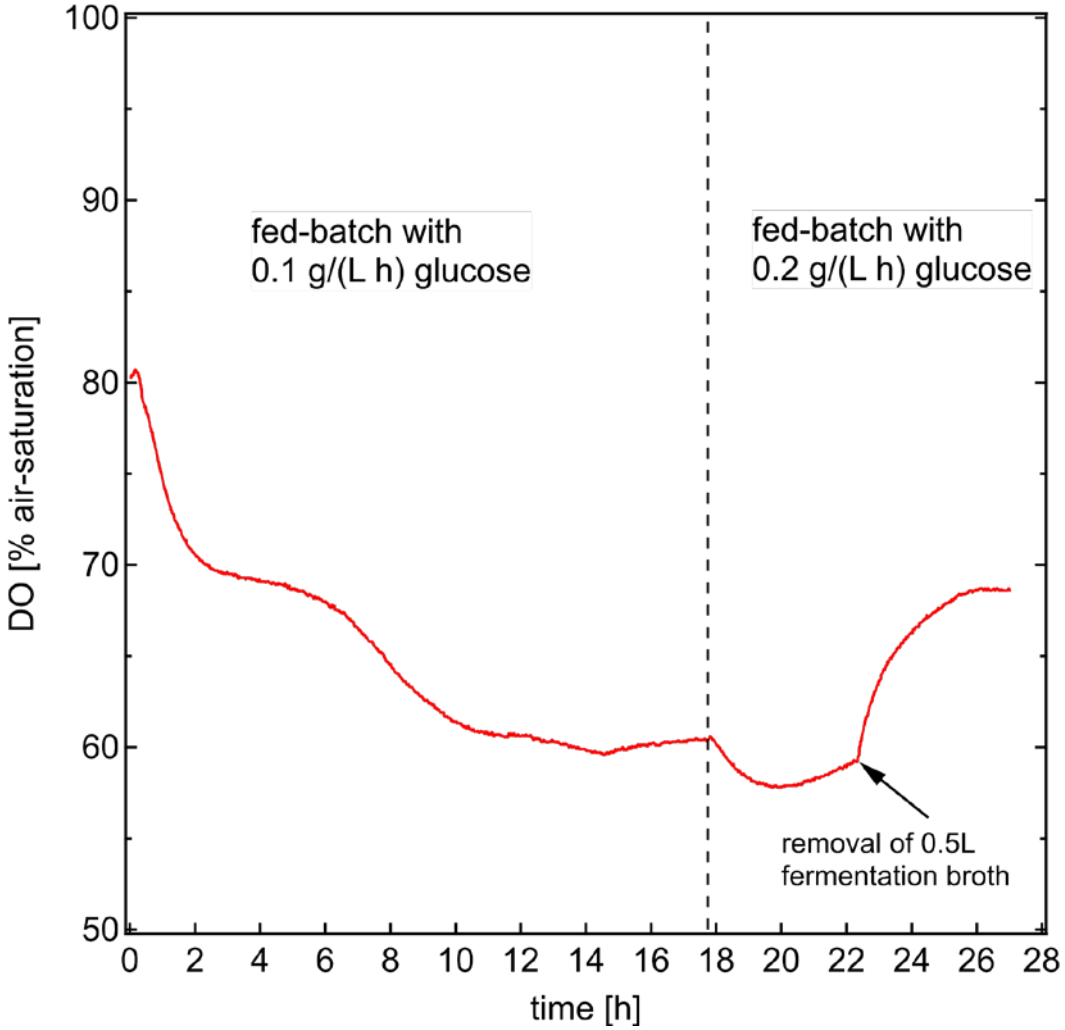

Fig. S20. DO profiles for an axenic *L. pentosus* culture growing on glucose in a membrane-aerated bioreactor (thin membrane, high OTR). In the first phase, a fed batch fermentation with a glucose feed of 100 $mgL^{-1}h^{-1}$ was performed followed by a second phase starting after 18 h where the glucose feed was doubled. After 22 h approximately 0.5 L of fermentation broth was removed from the reactor, which increased the k_La and thus led to an increase of the DO signal.

Tab. S1. Overview of pathways. *Lactobacillus pentosus* assimilates hexoses through the Embden-Meyerhof-Parnas pathway (1 mol glucose to 2 mol lactate) and pentoses through the phosphoketolase (PK) pathway (1 mol xylose to 1 mol lactic acid and 1 mol acetic acid). *Lactobacillus brevis* assimilates both hexoses (1 mol glucose to 1 mol lactic acid, 1 mol of acetic acid and 1 mol of carbon dioxide) and pentoses through the PK pathway. *Veillonella criceti* assimilates lactic acid to propionic and acetic acid. *Clostridium tyrobutyricum* can assimilate glucose to butyric acid and carbon dioxide. If *C. tyrobutyricum* co-produces butyric and acetic acid from glucose using electron bifurcation, the energy gain is with 3.25 mol ATP per mol glucose higher in comparison to sole butyric acid formation (3.0 mol ATP per mol glucose). *C. tyrobutyricum* assimilates 3 mol lactate and 1 mol of acetate to 2 mol butyric acid with carbon dioxide and hydrogen formation. *A. woodii* can utilize 4 mol lactate and 4 mol carbon dioxide to produce 6 mol acetate and 4 mol carbon dioxide. $\Delta_rG°$ values indicating the Gibbs free energy change in the forward direction at neutral pH and standard state conditions, which were obtained from the eQuilibrator website (*58*).

Lactobacillus pentosus and Lactobacillus brevis

Homofermentative glucose fermentation to lactic acid

$C_6H_{12}O_6 + 2\ ADP + 2P_i \rightarrow 2\ C_3H_6O_3 + 2\ H_2O + 2\ ATP$ $\Delta_rG'° = -135.8$ [kJ/mol]

Heterofermentative glucose fermentation to lactic and acetic acid

$C_6H_{12}O_6 + O_2 + 2\ ADP + 2\ P_i \rightarrow C_3H_6O_3 + CH_3COOH + CO_2 + 3\ H_2O + 2\ ATP$ $\Delta_rG'° = -633.5$ [kJ/mol]

Heterofermentative pentose fermentation to lactic and acetic acid

$C_5H_{10}O_5 + 2\ ADP + 2\ P_i \rightarrow C_3H_6O_3 + CH_3COOH + 2\ H_2O + 2\ ATP$ $\Delta_rG'° = -158.5$ [kJ/mol]

Veillonella criceti

Lactic acid fermentation to propionic and acetic acid

$3\ C_3H_6O_3 + ADP + P_i \rightarrow 2\ C_2H_5COOH + CH_3COOH + CO_2 + 2\ H_2O + ATP$ $\Delta_rG'° = -158.1$ [kJ/mol]

Clostridium tyrobutyricum

Glucose fermentation to butyric acid

$C_6H_{12}O_6 + 3\ ADP + 3\ P_i \rightarrow C_4H_8O_2 + 2\ CO_2 + 2\ H_2 + 3\ H_2O + 3\ ATP$ $\Delta_rG'° = -135.1$ [kJ/mol]

Glucose fermentation to butyric and acetic acid fermentation with electron bifurcation

$C_6H_{12}O_6 + 3.25\ ADP + 3.25\ P_i \rightarrow 0.75\ C_4H_8O_2 + 0.5\ CH_3COOH + 2\ CO_2 + 2.5\ H_2 + 3.25\ H_2O + 3.25\ ATP$ $\Delta_rG'° = -106.4$ [kJ/mol]

Lactic and acetic acid co-fermentation to butyric acid

$3\ C_3H_6O_3 + CH_3COOH + ADP + P_i \rightarrow 2\ C_4H_8O_2 + H_2O + 3\ CO_2 + 2\ H_2 + ATP$ $\Delta_rG'° = -56.2$ [kJ/mol]

Acetobacterium woodii

Homofermentative lactic acid fermentation to acetic acid

$4\ C_3H_6O_3 + 4\ CO_2 + 1.5\ ADP + 1.5\ P_i \rightarrow 6\ CH_3COOH + 4\ CO_2 + 1.5\ H_2O + 1.5\ ATP$ $\Delta_rG'° = -205.0$ [kJ/mol]

Trichoderma reesei

Respiration glucose

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ $\Delta_rG'° = -2930.4$ [kJ/mol]

Respiration xylose

$C_5H_{10}O_5 + 5\ O_2 \rightarrow 5\ CO_2 + 5\ H_2O$ $\Delta_rG'° = -2455.3$ [kJ/mol]

Tab. S2. CBP on different substrates in fed-batch mode by *T. reesei*, different LAB and *C. tyrobutyricum*.

Microbial consortium	Fig.	OTR	Substrate	Fermentation time [a] [h]	Acetic acid [a] [gL^{-1}]	Propionic acid [a] [gL^{-1}]	Butyric acid [gL^{-1}]	total carboxylic acids [a] [gL^{-1}]	Butyric acid yield [g/g fermentable sugars]	Product selectivity [b] [%]	Carboxylic acids yield [g/g fermentable sugars]	Productivity [g butyric acid L^{-1} h^{-1}]
	Fig. 2A	high	1.75 % (w/w) Avicel+9.32 gL^{-1} xylose [d]	166.92	0.3±0.1	0.0±0	4.1±0.8	4.4±0.8	0.14±0.03	93.1±0.8	0.15±0.03	0.02
T. reesei, *L. pentosus*, *C. tyrobutyricum*	**Fig. 2B**	high	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose + 2 gL^{-1} acetic acid [d]	216.92	3.1±0.1	0.0±0	10.7±0.2	13.8±0.2	0.37±0.01	77.6±0.3	0.41±0.01c	0.05
	Fig. 2D	low	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose [d]	371.67	0.4±0.1	0.0±0	10.2±0.5	10.5±0.5	0.35±0.02	96.7±0.1	0.37±0.02	0.03
T. reesei, *L. pentosus*, *L. brevis*, *C. tyrobutyricum*	**Fig. 2C**	high	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose [d]	202	2.7±0.1	0.0±0	9.5±0.4	12.1±0.3	0.33±0.01	77.6±0.6	0.42±0.01	0.05
T. reesei, *L. pentosus*, *C. tyrobutyricum*	**Fig. S10**A	low	3 % (w/w) pretreated beechwood with prehydrolyzate [f]	765	0.7±0.1	0.2±0.1	9.5±0.2	10.4±0.1	0.38±0.01	91.5±0.8	0.42±0.01	0.01
C. tyrobutyricum SSF, 15 FPU Accellerase © 1500 / g cellulose	**Fig. S7**	-	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose [g]	447.5	1.9±0.2	0.0±0	8.02±0.4	9.9±0.3	0.28±0.01	81±2.1	0.34±0.01	0.02

[a] At highest butyric acid concentration.
[b] Percent of butyric acid from total carboxylic acids.
[c] Fed acetic acid subtracted.
[d] Xylose or xylose/acetic acid fed with a constant feeding rate over 75 hours.
[e] Xylose fed with a constant feeding rate over 150 hours.
[f] Prehydrolyzate fed with a constant feeding rate over 200 hours.
[g] Avicel and 4.7 gL^{-1} xylose initially introduced and the remaining xylose fed with a constant feeding rate over 37.2 hours.

Tab. S3. Metabolic activity of *V. criceti* at 37 °C after 72.1 and 222.3 hours of cultivation under anoxic conditions for various media supplements. *V. criceti* is known for its high nutrient requirements, such as yeast extract and peptone. Since these complex nutrients are expensive and hamper a potential industrial application for the production of bulk chemicals, we tested if *V. criceti* grew on Mandels medium with only 0.75 gL^{-1} peptone and 0.25 gL^{-1} yeast extract as complex components. Metabolic activity could be measured (data not shown). The projected integration of the obligate anaerobe in the consortium would result in a nutritional competition. We added complex media components to the CBP lactate broth and tested their effect on the metabolic activity of *V. criceti*. The bacterium *V. criceti* grew on the lactate broth and converted 22.7±0.4 gL^{-1} lactic acid to 9.2±0.2 gL^{-1} propionic acid and 6.3±0.2 gL^{-1} acetic acid after 222.3 hours. Nevertheless, a beneficial impact of the supplementation, especially of trypticase peptone, was observed, reducing the fermentation time from 222.3 to 72.1 hours. Concentrations used: lactate broth, 274 mM; tryticase peptone, 5 gL^{-1}; yeast extract, 3 gL^{-1}. A "+" indicates supplementation. For all experiments, 0.75 gL^{-1} sodium thioglycolate was added.

	Lactate broth	Trypticase peptone	Yeast extract	Lactic acid utilized	Acetic acid produced	Propionic acid produced
				[mM]	[mM]	[mM]
72.1 hours	+	-	-	175.0 ± 67.8	44.8 ± 31.3	70.0 ± 33.0
	+	+	-	253.6 ± 2.1	88.3 ± 2.0	115.0 ± 3.8
	+	-	+	174.9 ± 4.9	46.8 ± 4.8	64.8 ± 1.2
	+	+	+	266.1 ± 6.1	98.6 ± 4.3	124.7 ± 0.1
222.3 hours	+	-	-	252.1 ± 4.8	79.9 ± 1.0	121.2 ± 2.9
	+	+	-	253.6 ± 2.1	86.2 ± 1.2	122.8 ± 3.3
	+	-	+	222.8 ± 3.3	71.2 ± 2.1	121.3 ± 6.3
	+	+	+	266.1 ± 6.1	93.0 ± 0.3	129.2 ± 2.5

Tab. S4. SCFA yields with lactate platform and alternative approaches.

Target product(s)	Microorganism	Feedstock	Process	Conc. [10]	Selectivity	Yield	Productivity	Lit.
				[gL^{-1}]	[%]	[gg^{-1}]	[$gL^{-1}h^{-1}$]	
Butyric acid	Mixed natural anaerobic consortium	NaOH pretreated rice straw	CBP	15.1	60.9	0.19 [1]	1.68-1.90	(*34*)
	C. thermocellum + *C. thermobutyricum*	NaOH pretreated rice straw	CBP	33.9	78	0.19 [1]	0.05	(*35*)
	Thermobifida fusca	Cellulose	CBP	2.1	n.d.	0.19 [1]	0.04	(*36*)
	LA Platform[2] + *C. tyrobutyricum*	**Steam pretreated beechwood**	**CBP**	**9.5**	**91.5**	**0.38 [1]**	**0.01**	**This study**
	GMO *C. tyrobutyricum*[4]	Glucose	Fed-batch	46.8	93	0.39 [1]	0.83	(*59*)
	GMO *E. coli*	Glucose, acetate	Fed-batch	10	99	0.50[6]	0.20	(*60*)
	C. tyrobutyricum[4]	Corn straw hydrolysate	Batch	29.6	84	0.32 [1]	0.49	(*59*)
	Clostridium sp S1[5]	Softwood hydrolysate	Batch	21.2	96	0.45 [7]	0.66	(*38*)
	Sequential LA Platform[2] + *C. tyrobutyricum*	**Glucose, acetate**	**Seq. batch**	**10.3**	**92**	**0.53 [8]**	**0.06**	**This study**
Mixed SCFAs	MixAlco	Municipal solid waste and sewage sludge	Four-stage countercurrent system fermentation	26	n.d.	0.18 [9]	0.06	(*61*)
	MixAlco	Lime pretreated corn stover and pig manure	Four-stage countercurrent system fermentation	16	n.d.	0.55 [9]	0.04	(*62*)
	M. elsdenii NCIMB 702410	Glucose	Batch	5.2	n.d.	0.44 [1]	0.03	(*63*)
	M. elsdenii NCIMB 702410	Corn stover hydrolyzate [11]	Batch	6.1	n.d.	0.43 [1]	0.04	(*63*)
	LA platform[2] + M. elsdenii	**Steam pretreated beechwood**	**Fed-batch**	**15.6**	**n.d.**	**0.42 [1]**	**0.01**	**This study**

[1] Yield in gg^{-1} (fermentable sugars added to the fermentation);
[2] Lactate Platform with *L. pentosus*;
[3] CBP to lactate, subsequent batch fermentations to acetate and butyrate;
[4] engineered to improve product selectivity;
[5] isolated strain able to consume acetate;
Yield in gg^{-1} (glucose and acetic acid): [6] 0.36, [7] 0.42, [8] 0.43;
[9] g carboxylic acids / g volatile solids digested;
[10] Concentration of total SCFAs as indicated in the target product column;
[11] deacetylated, dilute-acid pretreated, enzymatically hydrolyzed corn stover.

Tab. S5. CBP on different substrates in fed-batch mode by *T. reesei, L. pentosus* and *V. criceti*.

Microbial consortium	Fig.	OTR	Substrate	Fermentation time [h]	Acetic acid [gL^{-1}]	Propionic acid [gL^{-1}]	Total carboxylic acids [gL^{-1}]	Acetic acid yield [g/g fermentable sugars]	Propionic acid yield [g/g fermentable sugars]	Carboxylic acids yield [g/g fermentable sugars]	Productivity [g SCFAs L^{-1} h^{-1}]
T. reesei, L. pentosus, V. criceti	Fig. **S8**	low	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose [a]	371.7	4.5±0.2	4.4±0.2	8.9±0.1	0.16±0.01	0.15±0.01	0.31±0.01	0.02
	Fig. 3A	low	3.50 % (w/w) Avicel + 18.64 gL^{-1} xylose [b]	342.5	13.7±0.3	9.3±0.2	23.0±0.1	0.24±0.01	0.16±0.01	0.40±0.01	0.07
	Fig. S10B	low	3 % (w/w) pretreated beechwood with prehydrolyzate [c]	406	6.7±0.2	5.6±0.1	12.4±0.1	0.27±0.01	0.23±0.01	0.50±0.01	0.03

[a] Xylose fed at a constant rate over 75 hours.
[b] Xylose fed at a constant rate over 150 hours.
[c] Prehydrolyzate fed at a constant feeding rate over 200 hours.

Tab. S6. CBP on different substrates in fed-batch mode by *T. reesei, L. pentosus, M. elsdenii, C. tyrobutyricum* and *V. criceti*.

Microbial consortium	Fig.	OTR	Substrate	Fermentation time [h]	Acetic acid [gL^{-1}]	Propionic acid [gL^{-1}]	Butyric acid [gL^{-1}]	Valeric acid [gL^{-1}]	Caproic acid [gL^{-1}]	Total carboxylic acids [gL^{-1}]	Carboxylic acids yield [g/g fermentable sugars]	Productivity [g SCFAs L^{-1} h^{-1}]
T. reesei, L. pentosus, M. elsdenii	**Fig. 3B**	high	1.75 % (w/w) Avicel + 9.32 gL^{-1} xylose [a]	280	4.3±0.8	1.2±0.3	5.0±0.4	1.5±0.4	1.3±0.1	13.3±0.1	0.46±0.01	0.05
	Fig. S10C	low	3.86 % (w/w) pretreated beechwood with prehydrolyzate [b]	1080	6.8±0.4	1.0±.0.4	4.2±0.6	1.2±0.1	2.4±0.7	15.6+0.2	0.42±0.02	0.01
T. reesei, L. pentosus, M. elsdenii, C. tyrobutyricum	**Fig. S10D**	low	3.86 % (w/w) pretreated beechwood with prehydrolyzate [b]	1080	4.5	1	7.4	1.4	2.5	16.7	0.46	0.01
T. reesei, L. pentosus, M. elsdenii, V. criceti	**Fig. S10E**	low	3.86 % (w/w) pretreated beechwood with prehydrolyzate [b]	958.5	2.6	0.8	6.7	3.5	2.1	15.7	0.42	0.02

[a] Xylose fed at a constant rate over 75 hours.
[b] Prehydrolyzate fed at a constant feeding rate over 200 hours.

Tab. S7. Theoretical yields of fermentation via lactate platform compared to alternative direct sugar fermentation routes for selected target products. Shown are the yields for conversion of glucose, xylose, acetic acid and steam pretreated beechwood (a mixture of the three former compounds). The routes are described in **Tab. S8** and **Tab. S9**.

Route	**Target product**	**$Y_{P/Glu}$ [g/g]**		**$Y_{P/Xyl}$ [g/g]**		**$Y_{P/AA}$ [g/g]**		**$Y_{P/(Glu+Xyl+AA)}$ [g/g]**	
		LA Platform	Alternative	LA Platform	Alternative	LA Platform	Alternative	LA Platform	Alternative
1	Acetic acid	1.00	0.67	1.00	0.67	1.00	1.00	1.00	0.69
2	Acetic acid	0.22	0.22	0.53	0.53	1.00	1.00	0.35	0.35
	Propionic acid	0.55	0.55	0.33	0.33	0.00	0.00	0.46	0.46
3	Acetic acid	0.00	0.00	0.27	0.00	1.00	1.00	0.00	0.07
	Butyric acid	0.53	0.49	0.39	0.49	0.00	0.00	0.52	0.45
4	Poly-Hydroxybutyric acid	0.52	0.48	0.51	0.48	0.48	0.48	0.51	0.48
5	Caproic acid	0.43	0.43	0.39	0.39	0.00	0.00	0.47	0.47
6	Tripalmitin	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
7	Acetic acid	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.07
	Ethanol	0.77	0.51	0.77	0.51	0.77	0.00	0.77	0.48
8	Acetic acid	0.00	0.00	0.27	0.00	1.00	1.00	0.00	0.07
	Butanol	0.45	0.41	0.33	0.41	0.00	0.00	0.44	0.38
9	Acetic acid	0.33	0.33	0.60	0.33	1.00	1.00	0.45	0.38
	1.2-Propanediol	0.42	0.42	0.25	0.42	0.00	0.00	0.35	0.39
10	Acetic acid	0.33	unkn.	0.60	unkn.	1.00	unkn.	0.45	unkn.
	Propionic acid	0.21	unkn.	0.12	unkn.	0.00	unkn.	0.17	unkn.
	Propanol	0.17	unkn.	0.10	unkn.	0.00	unkn.	0.14	unkn.

Tab. S8. Overview of microorganisms and pathways used for the calculation of the theoretical yield of the lactate platform and conventional production routes using glucose as substrate.

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
1	I) Glucose --> 2 Lactic acid II) 2 Lactic acid --> 3 Acetic acid ---- Glucose --> 3 Acetic acid	*I) L. pentosus* *II) A. woodii*		I) Glucose --> 2 EtOH + 2 CO_2 II) 2 EtOH + 2 O_2 --> 2 Acetic acid + 2 H_2O ---- Glucose + 2 O_2 --> 2 Acetic acid + 2 CO_2 + 2 H_2O	*I) Saccharomyces cerevisiae* *II) Gluconobacter oxydans*	
2	I) 1.5 Glucose --> 3 Lactic acid II) 3 Lactic acid --> 2 Propionic acid + Acetic acid + CO_2 + H_2O ---- 1.5 Glucose --> 2 Propionic acid + Acetic acid + CO_2 + H_2O	*I) L. pentosus* *II) V. criceti*		1.5 Glucose --> 2 Propionic acid + Acetic acid + CO_2 + H_2O	*Propionibacterium* spp.	
3	I) 11 Glucose --> 22 Lactic acid II) 4 Lactic acid --> 6 Acetic acid III) 18 Lactic acid + 6 Acetic acid --> 12 Butyric acid + 18 CO_2 + 12 H_2 + 6 H_2O ---- 11 Glucose --> 12 Butyric acid + 18 CO_2 + 12 H_2 + 6 H_2O	*I) L. pentosus* *II) A. woodii* *III) C. tyrobutyricum*		Glucose --> Butyric acid+ 2 H_2 + 2 CO_2	*C. tyrobutyricum*	
4	I) 11 Glucose --> 22 Lactic acid II) 4 Lactic acid --> 6 Acetic acid III) 18 Lactic acid + 6 Acetic acid --> 12 Butyric acid + 18 CO_2 + 12 H_2 + 6 H_2O IV) 12 Butyric acid + 6 O_2 + 12 H_2O --> 12 Polyhydroxybutyrate + 24 H_2O ---------- 11 Glucose + 6 O_2 + 12 H_2O --> 12 Polyhydroxybutyrate + 18 CO_2 + 12 H_2 + 30 H_2O	I) L. pentosus II) A. woodii *III) C. tyrobutyricum* *IV) Rhodospirillum rubrum*	(*64*)	Glucose + 1.5 O_2 --> Polyhydroxybutyrate + 2 CO_2 + 3 H_2O	*Rhodospirillum rubrum*	(*64*)

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
5	I) 3 Glucose --> 6 Lactic acid II) 6 Lactic acid --> 2 Caproic acid + 6 CO_2 + 2 H_2O + 4 H_2 --- 3 Glucose --> 2 Caproic acid + 6 CO_2 + 2 H_2O + 4 H_2	*I) L. pentosus* *II) Megasphaera elsdenii*		3 Glucose --> 2 Caproic acid + 6 CO_2 + 2 H_2O + 4 H_2	*M. elsdenii*	(*63*)
6	I) 16.33 Glucose --> 32.67 Lactic acid II) 32.67 Lactic acid --> 49 Acetic acid III) 49 Acetic acid + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2 ---- 16.33 Glucose + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2	*I) L. pentosus* *II) A. woodii* *III) Yarrowia lipolytica*	(*65*, *66*) (Tripalmitin was used as model lipid.)	16.33 Glucose + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2	*Yarrowia lipolytica*	
7	I) Glucose --> 2 Lactic acid II) 2 Lactic acid --> 3 Acetic acid III) 3 Acetic acid + 6 CO + 3 H_2O --> 3 EtOH + 6 CO_2 ---- Glucose + 6 CO + 3 H_2O --> 3 EtOH + 6 CO_2	*I) L. pentosus* *II) A. woodii* *III) P. furiosus*	Engineered *P. furiosus* (*53*)	Glucose --> 2 EtOH + 2 CO_2	*S. cerevisiae*	
8	I) 11 Glucose --> 22 Lactic acid II) 4 Lactic acid --> 6 Acetic acid III) 12 Lactic acid + 6 Acetic acid --> 12 Butyric acid + 18 CO_2 + 12 H_2 + 6 H_2O IV) 12 Butyric acid + 24 CO + 12 H_2O --> 12 n-Butanol + 24 CO_2 ---- 11 Glucose + 24 CO + 12 H_2O --> 12 n-Butanol + 42 CO_2 + 12 H_2 + 6 H_2O	*I) L. pentosus* *II) A. woodii* *III) C. tyrobutyricum* *IV) P. furiosus*	Engineered *P. furiosus* (*53*)	Glucose --> n-Butanol + 2 CO_2 + H_2O	*C. acetobutyricum*	
9	I) Glucose --> 2 Lactic acid II) 2 Lactic acid --> 1,2-Propanediol + Acetic acid + CO_2 ---- Glucose --> 1,2-Propanediol + Acetic acid + CO_2	*I) L. pentosus* *II) L. buchneri*	(*67*)	Glucose --> 1,2-Proanediol + Acetic acid + CO_2	*C. thermosaccharolyticum*	(*68*)

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
10	I) Glucose --> 2 Lactic acid II) 2 Lactic acid --> 1.2-Propanediol + Acetic acid + CO_2 III) 1.2-Proanediol --> 0.5 1-Propanol + 0.5 Propionic acid + 0.5 H_2O ---- Glucose --> 0.5 1-Propanol + 0.5 Propionic acid + 0.5 H_2O + Acetic acid + CO_2	*I) L. pentosus* *II) L. buchneri* *III) L. diolivorans*	(*67*, *69*)			

Tab. S9. Overview of microorganisms and pathways used for the calculation of the theoretical yield of the lactate platform and conventional production routes using xylose as substrate.

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
1	I) Xylose --> Lactic acid + Acetic acid II) Lactic acid --> 1.5 Acetic acid ---- Xylose --> 2.5 Acetic acid	*I) L. pentosus* *II) A. woodii*		I) 3 Xylose --> 5 EtOH + 5 CO_2 II) 5 EtOH + 5 O_2 --> 5 Acetic acid + 5 H_2O ---- 3 Xylose + 5 O_2 --> 5 Acetic acid + 5 H_2O	*I) Pichia stipitis* *II) Gluconobacter oxydans*	
2	I) 3 Xylose -->3 Lactic acid + 3 Acetic acid II) 3 Lactic acid --> 2 Propionic acid + Acetic acid + CO_2 + H_2O --- 3 Xylose -->2 Propionic acid + 4 Acetic acid + CO_2 + H_2O	*I) L. pentosus* *II) V. criceti*		3 Xylose --> 2 Propionic acid + 4 Acetic acid + CO_2 + H_2O	*Propionibacterium* spp.	
3	I) 3 Xylose --> 3 Lactic acid + 3 Acetic acid II) 3 Lactic acid + Acetic acid --> 2 Butyric acid + 3 CO_2 + 2 H_2 + H_2O ---- 3 Xylose --> 2 Butyric acid + 2 Acetic acid + 3 CO_2 + 2 H_2 + H_2O	*I) L. pentosus* *II) C. tyrobutyricum*		3 Xylose --> 2.5 Butyric acid + 5 H_2 + 5 CO_2	*C. tyrobutyricum*	
4	I) 3 Xylose --> 3 Lactic acid + 3 Acetic acid II) 3 Lactic acid + Acetic acid --> 2 Butyric acid + 3 CO_2 + 2 H_2 + H_2O III) 2 Butyric acid + O_2 + 2 H_2O --> 2 PHB + 4 H_2O IV) 2 Acetic acid + O_2 --> 0.667 Polyhyydroxybutyrate + 1.33 CO_2 + 2 H_2O ---- 3 Xylose + 2 O_2 + 2 H_2O --> 2.667 Polyhydroxybutyrate + 4.33 CO_2 + 2 H_2 + 7 H_2O	I) L. pentosus *II) C. tyrobutyricum* III-IV) *Rhodospirillum rubrum*	(*64*)	1.2 Xylose + 1.5 O_2 --> Polyhydroxybutyriate + 3 H_2O + 2 CO_2	*Burkholderia sacchari*	(*70*)

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
5	I) 2 Xylose --> 2 Lactic acid + 2 Acetic acid II) Lactic acid + Acetic acid --> Butyric acid + H_2O + CO_2 III) Lactic acid + Butyric acid --> Caproic acid + H_2O + CO_2 ---- 2 Xylose --> Caproic acid + Acetic acid + $2H_2O$ + 2 CO_2	I) *L. pentosus* II-III) *Megasphaera elsdenii*		2 Xylose --> Caproic acid + Acetic acid + 2 H_2O + 2 CO_2	*M. elsdenii*	
6	I) 19.6 Xylose --> 19.6 Lactic acid + 19.6 Acetic acid II) 19.6 Lactic acid --> 29.4 Acetic acid III) 49 Acetic acid + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2 ---- 16.33 Glucose + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2	*I) L. pentosus* *II) A. woodii* *III)* e.g. *Yarrowia lipolytica*	(*65*, *66*) (Tripalmitin was used as model lipid.)	16.33 Glucose + 13.5 O_2 --> $C_{51}H_{98}O_6$ + 47 CO_2	*Y. lipolytica*	
7	I) 2 Xylose --> 2 Lactic acid + 2 Acetic acid II) 2 Lactic acid --> 3 Acetic acid III) 5 Acetic acid + 10 CO + 5 H_2O --> 5 EtOH + 10 CO_2 ---- 2 Xylose + 10 CO + 5 H_2O --> 5 EtOH + 10 CO_2	*I) L. pentosus* *II) A. woodii* *III) P. furiosus*	Engineered *P. furiosus* (*53*)	3 Xylose --> 5 EtOH + 5 CO_2	*S. cerevisiae*	
8	I) 3 Xylose --> 3 Lactic acid + 3 Acetic acid II) 3 Lactic acid + Acetic acid --> 2 Butyric acid + 3 CO2 + 2 H2 + H2O III) 2 Butyric acid + 4 CO + 2 H_2O --> 2 n-Butanol + 4 CO_2 ---- 3 Xylose + 4 CO + 2 H_2O --> 2 n-Butanol + 2 Acetic acid + 7 CO_2 + 2 H_2 + H_2O	*I) L. pentosus* *II) C. tyrobutyricum* *III) P. furiosus*	Engineered *P. furiosus* (*53*)	6/5 Xylose --> n-Butanol + 2 CO_2 + H_2O	*C. acetobutyricum*	
9	I) 2 Xylose --> 2 Lactic acid + 2 Acetic acid II) 2 Lactic acid --> 1,2-Propanediol + Acetic acid + CO_2 ---- 2 Xylose --> 1,2-Propanediol + 3 Acetic acid + CO_2	*I) L. pentosus* *II) L. buchneri*	(*67*)	6/5 Xylose --> 1,2-Propanediol + Acetic acid + CO_2	*C. thermosaccharolyticum*	(*68*)

Route	LA Platform	Microorganism(s)	Literature / Comments	Conventional Approach	Microorganism(s)	Literature / Comments
10	I) 2 Xylose --> 2 Lactic acid + 2 Acetic acid II) 2 Lactic acid --> 1,2-Propanediol+ Acetic acid + CO_2 III) 1,2-Proanediol --> 0.5 1-Propanol + 0.5 Propionic acid + 0.5 H_2O ---- 2 Xylose --> 0.5 1-Propanol + 0.5 Propionic acid + 0.5 H_2O + 3 Acetic acid + CO_2	*I) L. pentosus* *II) L. buchneri* *III) L. diolivorans*	(*67*, *69*)			