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FULL PAPER

Chemoenzymatic 1,3-butadiene synthesis from syngas using biological decarboxylative Claisen condensation and zeolite-based dehydration

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Supporting information for this article is given via a link at the end of the document.

Abstract: A method for producing 1,3-butadiene (1,3-BD) by an amalgamation of chemical and biological approach involving syngas as the carbon source is proposed here. Syngas is converted to the central intermediate, acetyl-CoA, through microorganisms having a tetrahydrofolate metabolism pathway. Acetyl-CoA is subsequently converted to malonyl-CoA using a carbonyl donor in the presence of a carboxylase enzyme. A decarboxylative Claisen condensation of the malonyl-CoA and acetaldehyde ensues in the presence of acyltransferases to form 3-hydroxybutyryl-CoA that is subsequently reduced by aldehyde reductase to obtain 1,3-butanediol (1,3-BDO). An ensuing dehydration step converts 1,3-BDO to 1,3-BD in the presence of a chemical dehydrating reagent.

Introduction

Synthesis gas (syngas) a mixture of hydrogen (H_2), carbon dioxide (CO_2) and carbon monoxide (CO), is a waste gas product in the chemical and biorefining industries that has found a vast number of applications.[1,2] Besides being used as a fuel, syngas can be converted into value added chemicals viz. alkanes,[3] olefins,[4] oxygenates, and alcohols[5] Carbon is fixed in anaerobic microorganisms from gaseous substrates like CO or CO_2 via the Wood-Ljungdahl (WL) pathway.[6] CO from syngas is converted to the central intermediate, acetyl-CoA, through the WL pathway utilizing tetrahydrofolate metabolism. Acetyl-CoA serves as the key building block to synthesize several products like ethanol and isopropanol.[7]

1,3-butadiene (1,3-BD) is an important industrial chemical obtained from the steam cracking process of petrochemical-based feedstocks and purified via extractive distillation.[8,9] The global annual production of 1,3-BD is about 10 million metric tons[10] and is applied in the manufacturing of polymers such as synthetic rubber,[11] ABS resins,[12] and chemicals such as hexamethylenediamine.[13] However, no biological process using microorganisms exists in nature for the production of this major petrochemical intermediate.[14] Given the vagaries of oil price,[15] dependency on the petrochemical feedstocks along with the increasing environmental concerns, there is a need for alternative, environmentally-friendly and sustainable process[16] for the production of 1,3-BD.[8] A partial mitigation to these issues would be to use an alternative approach utilizing bio-based feedstocks. Recently, by combining the usefulness of chemical and biological techniques, we demonstrated the successful synthesis of 1,3-BD from the key intermediate erythritol obtained from xylose via biomass deconstruction.[17]

In the current study, another unique approach for the production of 1,3-BD using combination of enzymatic and chemical reactions is described. A novel approach, where in vitro sequential enzymatic reactions to produce 1,3-butanediol (1,3-BDO) from common intracellular metabolites viz. acetaldehyde and malonyl-CoA has been demonstrated. The 1,3-BDO formed was successfully dehydrated chemically to produce 1,3-BD.

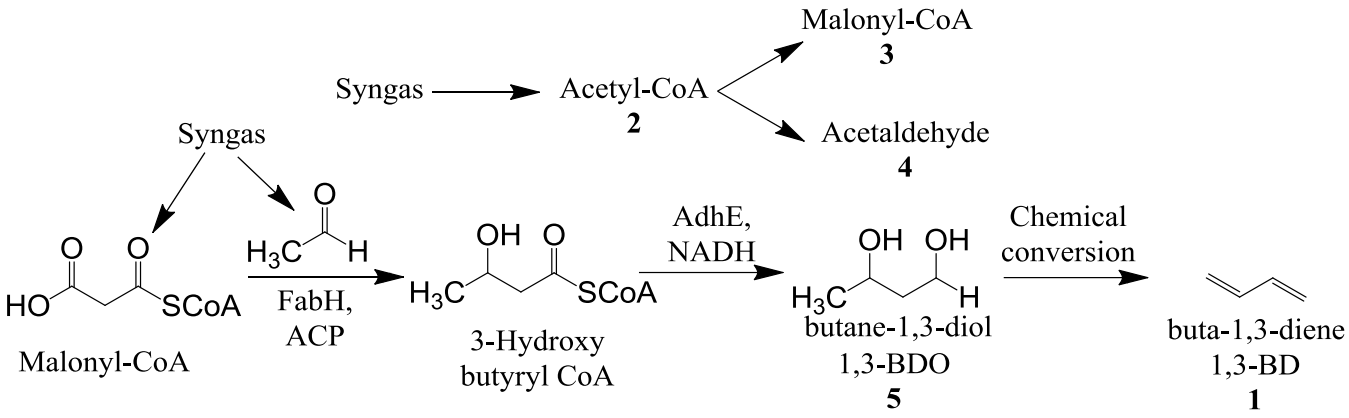
Results and Discussion

Proposed novel pathway

An alternative, environmentally-friendly and sustainable process for the production of 1,3-BD **1**, would mandate either a purely biological approach or a combination of biological and chemical routes. Several methodologies have been proposed for 1,3-BD production including (i) via enzymatic dehydration of butanediols[18] (ii) enzymatic dehydrogenation and dehydration of butanols[18] (iii) enzymatic hydroxylation and dehydration of butenes[18] (iv) from 2,4-pentadienoate[19] (v) from acetyl-CoA via crotonyl-CoA and crotyl alcohol.[20] Demonstration of in vitro production of 1,3-BD from crotyl alcohol using mutants of linalool dehydratase and alkenol dehydratase was reported by Genomatica and Braskem recently.[21] Most of these processes involves extensive genetic engineering to demonstrate proof of concept.

Evidently, improved versions of existing enzymes having promiscuous activity, suitable microbial host and efficient protocols forms the basis for producing 1,3-BD from various renewable feedstocks as well as C1 feedstocks such as syngas. The syngas utilising bacteria employ the WL pathway for assimilation of CO/CO_2 as carbon source along with H_2. The key central intermediate in the WL pathway is acetyl-CoA which can then be diverted via multitudes of routes to make various products of interest.

Towards this end, a novel route for 1,3-BD from syngas has been proposed in the current study using malonyl-CoA **3** and acetaldehyde **4** as starting materials, both of which can be derived from acetyl-CoA **2**, the key metabolite in the WL pathway.[6] Acetyl-CoA can be converted to malonyl-CoA using a carbonyl donor in the presence of a carboxylase enzyme. Decarboxylative Claisen condensation (DCC) of the malonyl-CoA and acetaldehyde will form 3-hydroxybutyryl-CoA in the presence of



Scheme 1. Novel route for synthesizing 1,3-butadiene from syngas

acyltransferases which can be reduced using a carboxylate reductase to obtain 1,3-BDO **5**. The generated 1,3-BDO can then be converted to 1,3-BD via a chemical process. The genesis of the envisaged pathway is based on the DCC reaction, hitherto not reported, between malonyl-CoA and acetaldehyde (Scheme 1).

Construction of recombinant plasmids and strains

As the DCC reaction between malonyl-CoA and acetaldehyde is the fundamental premise of our proposed strategy, exploring for enzymes having this promiscuous activity was crucial. One of the enzymes in the fatty acid pathway, FabH, which already catalyzes the condensation reaction between malonyl-CoA and acetyl-CoA appeared to be a probable candidate.[22] Therefore, exploiting this strategy, FabH encoded by the *fabH* gene along with the gene for the acyl carrier protein (ACP) was sourced from *E. coli*. For the subsequent aldehyde reductase (AdhE) step, multiple sources were evaluated. Subsequently, genes coding for AdhE's from *C. acetobutylicum* and *C. beijerinckii* were chosen.

The *fabH* and *acp* genes having a size of about 955 and 237 bp respectively were amplified from genomic DNA of *E. coli* K12. Similarly, different aldehyde-alcohol dehydrogenase (adhE) were amplified using *C. acetobutylicum* (Ca_adhE) and *C. beijerinckii* (Cbei_adhE) genomic DNA using One Taq polymerase with Tm 55 ˚C. PCR amplifications of *fabH*, *acp*, and different adhE's (Cbei_adhE) from *C. beijerinckii* are shown in Figure S1a, S2a, S3a, and S4a respectively. The genes were cloned into pET30a (+) vector with respective restriction enzymes mentioned in Table S1. Putative positives were analyzed by colony PCR as shown in Figure S1b, S2b, S3b, S4b for respective genes. The recombinant plasmid carrying the fabH, and acp genes were validated by the digesting with same restriction enzymes pairs i.e. *Bam*HI/*Hin*dIII and *Kpn*I/*Xho*I respectively. The different *adhE* genes carrying pET30a (+) recombinant vectors were also confirmed by restriction digestion using same pair of enzymes as mentioned in Table S1. *E. coli* DH5α strain was used for plasmid propagations and confirmed plasmid were transformed into *E. coli* BL21 (DE3) cells for expression analysis.

Expression and purification of His-tagged protein

The FabH, ACP and AdhE proteins expressed in *E. coli* BL21 (DE3) were purified on Ni-NTA and the SDS-PAGE of the samples confirmed >90 % purity. Purified protein bands of 14 kDa, 52 kDa and 35 kDa size corresponding to the enzymes ACP, AdhE and FabH respectively are shown in Figure S5.

Activity of FabH

Enzyme activity of purified FabH protein was performed using the substrates malonyl-CoA and acetaldehyde in the presence of purified ACP, FabH and cerulenin which inhibits any undesired chain elongation. Figure 1 depicts the HPLC analysis of the reaction mixture indicating a distinct peak of 3-hydroxybutyryl-CoA that com-pared well with the retention time of standard 3-hydroxybutyryl-CoA. Demonstration of the evidence that the *E. coli* FabH along with ACP can also carry out the DCC reaction with uncommon substrates malonyl-CoA and acetaldehyde is a major finding in this study. Successful proof of this activity opens up tremendous opportunity for FabH to be explored for condensation activities with other uncommon substrates leading to novel products besides its classical role.

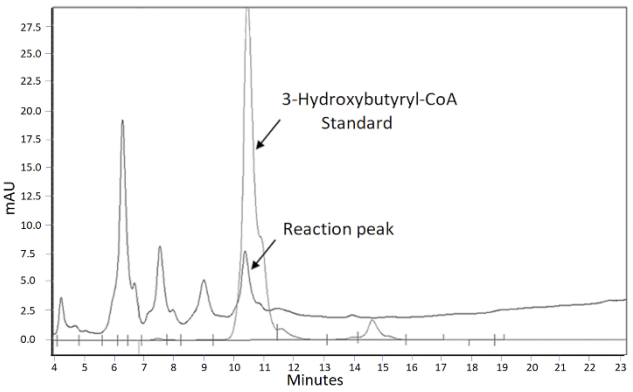


Figure 1. HPLC spectrum of 3-hydroxybutyryl-CoA obtained from FabH enzyme assay and overlaid with standard.

Cascade reaction with AdhE

Once the DCC reaction was successful between malonyl-CoA and acetaldehyde, the next step was to convert the product 3-hydroxybutyryl-CoA to 1,3-BDO. It is a well-known fact that CoA derivatives of compounds are highly unstable and not easy to isolate. Therefore, we relied on telescoping of the obtained 3-hydroxybutyryl-CoA for the subsequent reaction, to monitor the formation of the 1,3-BDO. The activities of FabH/ACP and AdhE were coupled together in presence of NADH in a cascade reaction. The production of 1,3-BDO was confirmed using HPLC (Figure 2 and Figure S6) and GC-MS (Figure S7). The mass

spectra showed the typical fragmentation pattern of 1,3-BDO with peaks at 43, 45 and 73 m/z values (Figure S8).

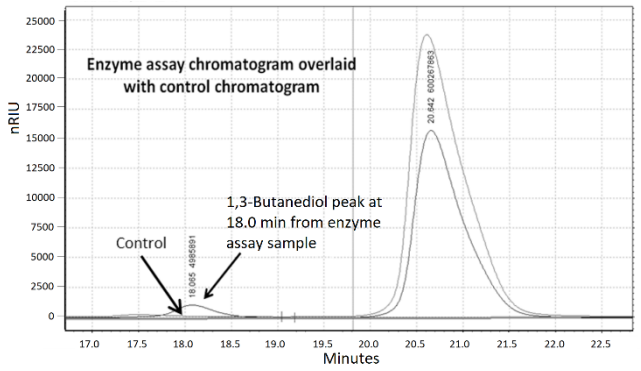
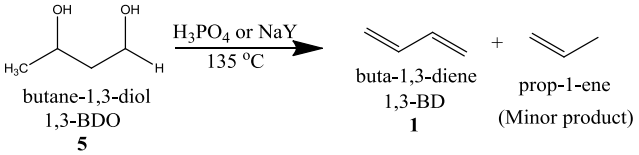


Figure 2. HPLC spectrum of 1,3-BDO (retention time ~18.05 min) obtained from cascade reaction (FabH/ACP and AdhE) and over-laid with standard 1,3-BDO.

Chemical conversion of 1,3-BDO to 1,3-BD

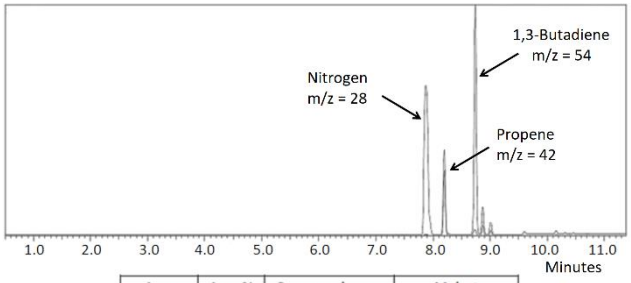
In the absence of any existing enzyme that can dehydrate a diol to diene, it was decided to convert the 1,3-BDO formed from the enzymatic reactions to 1,3-BD using chemical catalysis. Optimization of reaction conditions for the conversion of 1,3-BDO to 1,3-BD was carried out using commercially available 1,3-BDO. Double dehydration reaction of 1,3-BDO was carried out under heating condition in a sealed vial and head space was monitored for the formation of 1,3-BD by GC. Initial screening using mineral acids viz. HCl, HI, H_2SO_4, H_3PO_4 and zeolites viz. ZSM5 and NaY (faujasite Y) revealed that dehydration using 35% HCl, HI and ZSM5 were not effective as they converted only a small fraction of 1,3-BDO and produced 1,3-BD in miniscule quantities (1-2 % yield). Whereas, H_2SO_4, H_3PO_4 and NaY, produced 1,3-BD in measurable quantities of around 10 % yield (Scheme 2). Cognizant of the fact that the 1,3-BDO produced from the fermentation will be in aqueous layer, use of H_2SO_4 was avoided and reaction with aqueous compatible mineral acid H_3PO_4 and zeolite NaY was screened further. Taking into account, the volume of 1,3-BD that will have to be produced at commercial scale, conditions were optimized by varying several parameters including equivalence, temperature, and reaction time (Figure S9).



Scheme 2. Reaction scheme for synthesizing 1,3-BD from 1,3-BDO.

Screening using H_3PO_4

For screening, 1.0 g of 1,3-BDO was subjected to double dehydration reaction in a sealed vial and the progress of the reaction was monitored by analyzing the headspace gas using GC. The GC chromatogram indicated the presence of only two peaks, one appearing at RT of 5.5 min and the other at 5.9 min. Comparing the GC chromatogram with that of standard 1,3-BD revealed that the peak corresponding to RT of 5.9 min matched with that of the standard 1,3-BD. However, the peak at 5.5 min required further probing. GC-MS analysis revealed the presence of two major peaks, one of m/z= 42 and other of m/z=54, the latter corresponding to 1,3-BD; along with trace amount of other impurities (cis and trans butene) (Figure 3).



Area	Area %	Compound name	Mol wt
26478863	43.62	Nitrogen	28
8117997	13.37	Propene	42
22381628	36.87	1,3-Butadiene	54
2471759	4.07	2-Butene	56
1258725	2.07	2-Butene, (E)-	56

Figure 3. Mass spectrum of 1,3 BD obtained by reacting 1,3-BDO with H_3PO_4 and Zeolite NaY.

Based on the m/z results, it was speculated that the peak corresponding to the RT 5.5 min on GC could be propene. To confirm, sample from dehydration reaction of propanol to propene under similar conditions was analysed by GC. The RT and molecular weights obtained from this reaction matched perfectly with that of the peak obtained during the dehydration reaction of 1,3-BDO indicating presence of propene. The residual 1,3-BDO was analyzed using HPLC to determine the conversion rate. The results of the screening studies are given in supporting information (Figure S9a, b, c).

Based on the optimization studies, the best conversion was achieved by reacting 1,3-BDO (1 g) with 1 ml of H_3PO_4 at 105 °C for 24 h. The yields of 1,3-BD and propene were found to be 35% and 4% respectively based on standard graphs (Figure S11 and Figure S12).

Screening using Zeolite NaY

Synthetic zeolites are widely used as catalysts in the petrochemical industry for fluid catalytic cracking and hydrocracking. The acidic forms of zeolites facilitates reactions, such as isomerisation, alkyla-tion, and cracking.[23-25]

NaY (Faujasite Y), an acidic catalyst was calcinated prior to the reaction at 540 °C for 16 h to remove moisture and ligand and allowed to cool to ambient temperature under nitrogen flow in a desiccator. The free-flowing solid NaY was used as such for initial screening and was later stored in a desiccator. Initial screening was performed using 1.0 g of 1,3-BDO and NaY under heated condition in a sealed vial. The progress of the reaction was monitored using GC by injecting gas sample from the headspace of the vials. Similar to the results with H_3PO_4, two peaks were observed on GC corresponding to RT= 5.5 min (propene) and RT= 5.9 min (1,3-BD). Optimization of the reaction temperature, time and loading (Figure S9d,e and f), revealed that reacting 1,3-BDO with 500 mg of zeolite NaY at 135 °C for 24 h gave best conversion resulting in a yield of 24 % of 1,3-BD. Reaction yields were derived from the standard graphs (Figure S10 and Figure S11).

Using mixture of H_3PO_4 and Zeolite NaY

Despite the optimization, the conversion of 1,3-BDO to 1,3-BD did not exceed beyond 35 % and 24 % using H_3PO_4 and NaY

respectively. Therefore, a different strategy was attempted where a combination of H_3PO_4 and NaY was used. The stability of NaY under highly acidic conditions is not known, as there are no reports on the combination of both mineral acids and zeolites. Nevertheless, the best conditions obtained during screening studies were employed. Temperature screening experiment revealed that the conversion was extremely low at 105 °C. Hence, all further reactions were carried out at 135 °C and effect of varying the loadings of NaY and H_3PO_4 was evaluated.

Table 1 shows the yield of 1,3-BD at varying concentration of H_3PO_4 in a reaction containing 1,3-BDO (1 g) along with 500 mg of NaY at 135 °C for 8 h. The highest yield of 16 % 1,3-BD was obtained with 0.5 ml of H_3PO_4.

Table 1. Effect of different loadings of H_3PO_4 for reaction conducted at 135 °C for 8 h.

Reaction	Loading of H_3PO_4 (mL)	GC observation	
		Propene (5.5 min)	1,3-BD (5.9 min)
1 g 1,3-BDO + 500 mg NaY	0.25	~1%	12%
	0.5	~4%	16%
	0.75	~5%	14%
	1.0	~7%	12%

Further, effect of varying NaY loading was also tested in a reaction containing 1,3-BDO (1 g) along with 0.5 ml of H_3PO_4 at 135 °C for 8 h. The highest yield of 15 % 1,3-BD was obtained with 200 mg of NaY (Table 2).

Based on the results obtained from loading optimization studies, it was found that reacting 1,3-BDO (1 g) with a mixture of 0.5 ml of H_3PO_4 and 200 mg of zeolite NaY at 135 °C for 24 h gave best conversion of 64 % 1,3-BD. Reaction yields were derived from the standard graph of 1,3-BD (Figure S11).

Table 2. Effect of different loadings of NaY for reaction conducted at 135 °C for 8 h.

Reaction	Loading of NaY (mg)	GC observation	
		Propene (5.5 min)	1,3-BD (5.9 min)
1 g 1,3-BDO + 0.5 ml H_3PO_4	100	~1%	7%
	200	~1%	15%
	300	3%	12%
	400	5%	14%

There are several reports in the literature on dehydration of all types of butanediols to 1,3-BD by chemical catalysis. One important criteria cited is that although complete dehydration of these butanediols into BD is possible, the process needs higher reaction temperatures and results in the production of more byproducts.[26] A temperature of 200 °C or more is typical of these reactions with BD yields of < 42 %. With respect to conversion of 1,3-BDO to 1,3-BD per se, a maximum 1,3-BD yield of 60% was achieved at 300 °C over ZSM5 with a SiO_2/Al_2O_3 ratio of 260 with the simultaneous formation of propylene at a BD/propylene selectivity ratio of 2.5.[27] In the current study, at the batch scale, it was possible to get a 1,3-BD yield of 64 % by operating at 135 °C for 24 h using a combination of H_3PO_4 and zeolite NaY.

Scale up reaction based on fixed bed reactor

The next step was to translate the feasibility of the reaction carried out in sealed vials to a higher scale. One of the industrially scalable process is based on catalytic cracking.[28] Catalytic cracking uses reactor and a regenerator where feed is injected onto hot, fluidized catalyst and large gasoline molecules are broken into smaller gasoline molecules and olefins. The vapor-phase products are separated from the catalyst using nitrogen flow. To translate our results, we used fixed bed reactor for the conversion.

Scale up of the 1,3-BDO to 1,3-BD reaction described above was met with two major challenges: (i) very high viscosity of feed 1,3-BDO (98.3 cP) and (ii) preparing the extrude having the crush strength in the range of 1.8-2.0 Pa from the hitherto combination of zeolite NaY and H_3PO_4 to form the fixed bed. To circumvent the viscosity issues, 1,3-BDO was diluted with water and 20-30% solution was found to be optimum for pumping. To fix the crush strength, series of extrudes were prepared by mixing NaY and H_3PO_4 in H_2O to keep the NaY complex intact and using alumina as a binder. The concentration of H_3PO_4 was varied from 20 to 40 % and binder was varied from 10 to 50 % to improve the crush strength. A mixture of 40 % H_3PO_4 and 20 % binder had the required crush strength of 1.8 Pa.

For initial studies, the feed containing 20 % 1,3-BDO was pumped in to the reactor using peristaltic pump at a rate of 5-10 mL h^{-1}. The mixture was heated in the preheater between 240-270 °C, followed by pushing the stream into the reactor column containing the bed at 290-320 °C using nitrogen flow of 40-60 mL min^{-1}. Initial reaction was carried out for 4 h. The vent coming out of the reactor was connected to a liquid gas separator which was maintained at a temperature of 5-10 °C. The head space was analyzed using GC for the production of 1,3-BD and the liquid collected in the gas liquid separator was analyzed using HPLC for residual 1,3-BDO at hourly intervals. After the 1st hour of reaction, GC analysis of the sample revealed the formation of 1,3-BD (RT 6.0 min) along with propene (RT 5.5 min). The presence of 1,3-BD was also confirmed by GC-MS. The AUC values for 1,3-BD, propene and the 1,3-BDO was constant during the hourly analysis indicating a steady state. Post this, higher concentration of 1,3-BDO (30 %), preheated at 270 °C, was pumped into the reactor using peristaltic pump using nitrogen flow. The reactor bed was maintained at 310 °C and the reaction was carried out for 5.5 h. The outlet of the reactor was connected to the gas liquid separator which collects the liquid and the gas coming out was connected to the hood. The vent coming out of the reactor was analyzed for 1,3-BD every 100 min using GC by collecting the gas in a bladder for 10 min. The liquid collected in the gas liquid separator was analyzed using HPLC. The yields were calculated based on the observations from 3 trial runs. It was observed that, 3.5 g of 1,3-BDO was consumed in 100 min and 26.5 g of unreacted 1,3-BDO was recovered from gas liquid separator. Yield of 1,3-BD was found to be 88% and that of propene was 8.0% from the consumed 1,3-BDO as revealed by GC. The product was characterized and confirmed using ^{1}H-NMR (Figure S13). Thus, the catalytic double dehydration of 1,3-BDO to 1,3-BD was successfully demonstrated in a fixed bed reactor.

After the reaction, the catalyst was regenerated by purging air to remove the deposited coke, a byproduct in the cracking process from the surface of the catalyst. The regenerated catalyst is then circulated back to the reactor to complete its cycle.

Thus far, there is only one other study wherein a fixed bed reactor has been used for dehydration of 2,3-butanediol to 1,3-BD supported by a simulated one-dimensional heterogenous reactor model.[29]

Conclusion

A novel sustainable process for producing the commodity chemical 1, 3-BD using a combination of biological and chemical method is reported in the current study. The process involves producing 1,3-BDO using FabH enzyme for DCC using uncommon substrates viz. malonyl-CoA and acetaldehyde (both derived from the key metabolic intermediate acetyl-CoA in the WL pathway). 1,3-BDO is then converted into butadiene by a chemical dehydration step using a unique combination of mineral acid and zeolites. The biological process can easily be engineered into syngas fermenting organisms and hence provides for a sustainable method for production of 1,3-BD.

Experimental Section

General methods, plasmids and strains: Methods for restriction enzyme digestions, ligation, transformation, and other standard molecular biology manipulations were based on methods described elsewhere[30] or as suggested by the manufacturer. Protein was analyzed by polyacrylamide gel electrophoresis (PAGE) under either denaturing condition using sodium dodecyl sulfate (SDS) or native conditions on gels having 7.5-12% poly-acrylamide. The gels were stained with Coomassie brilliant blue. All oligonucleotides were purchased from Eurofins India. Table S1 in Supporting Information summarizes the primers used in this study. One Taq DNA polymerase, restriction enzymes and T4 DNA ligase were bought from New England Biolabs. DNA purification, plasmid isolation and PCR purification kits were purchased from Qiagen. Ni-NTA resin was bought from Qiagen and PD10 desalting columns from GE-Healthcare. All other reagents were of analytical grade. The pET-30a (+) plasmid and *E. coli* BL21 (DE3)/DH5α strains were used as expression vector and host strains and were procured from Invitrogen. The strains were propagated at 37 °C in Luria-Bertani (LB) medium containing 50 µg mL^{-1} kanamycin for selection with shaking at 220 rpm.

Expression and purification of His-tagged proteins: For expression tests, 10 mL of LB medium containing 50 µg mL^{-1} kanamycin was inoculated with a freshly isolated colony of the host strain (*E. coli* BL21(DE3)) carrying the recombinant plasmid of pET30a-FabH, pET30a-ACP, and pET30a-Cbei_3832 respectively. The inoculated cultures were incubated overnight at 37 ˚C and diluted 1:100 into 2 mL of fresh LB medium containing antibiotics with shaking until OD_{600} = 0.6; induced with IPTG at a final con-centration of 0.5 mM and were grown at 20 °C and 37 °C for 12 and 8 h respectively. 1 mL sample of induced cultures grown under different conditions were centrifuged and resuspended in 1 mL of lysis buffer. A portion was mixed with equal portion of 2X SDS loading buffer and boiled for 10 min to prepare whole-cell lysate for expression analysis on SDS-PAGE. The samples for FabH and AdhE was run on 12 % SDS-PAGE gel and ACP was run on 16% Tricine gel; gels were visualized by Coomassie Brilliant Blue R-250 staining. The target proteins were detected by comparison with protein standard markers. Optimum conditions for high expression were determined based on the observations from the SDS-PAGE gel (data not shown).

For purification of the protein, growth and expression were carried out in 500 mL scale in optimal conditions identified by the expression tests. The cells harvested from 500 mL of culture grown and induced under optimum conditions were suspended in 10 mL of lysis buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, and 5 mM imidazole) containing lysozyme (1 mg mL^{-1}) and cells were disrupted by sonication. Soluble and insoluble cell fractions were separated by centrifugation at 15000 rpm for 10 min in cold. Supernatants carrying the soluble fractions were mixed with 1 mL Ni-NTA resin to purify target proteins according to manufacturer's manual. Bound His-tagged proteins were eluted in 1 mL of elution buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, and 250 mM imidazole, at pH 8.0. Equal volume of purified proteins were mixed with 2 X SDS loading buffer and boiled for 10 min to prepare samples for SDS-PAGE. The samples for FabH and AdhE was run on 12 % SDS-PAGE gel and ACP was run on 16 % Tricine gel and the gels were visualized by Coomassie Brilliant Blue R-250 staining. The target proteins were detected by comparison with protein standard markers. The proteins were buffer exchanged into the assay buffer 50 mM NaH_2PO_4 (pH 8.0) using a prepacked PD-10 Sephadex G-25 desalting column.

Acyl carrier protein was also purified and used in the enzyme reaction mixture. The total protein content as estimated by bicinchoninic acid assay (BCA) method for FabH and AdhE was 200 mg L^{-1} and 15 mg L^{-1} respectively

FabH/ACP enzyme activity: Typical reaction mixture for determining FabH/ACP activity constituted of malonyl-CoA (200 µM), acetaldehyde (250 µM), purified ACP protein (200 µg) which was mixed thoroughly followed by addition of cerulenin (250 µM) (solution made in ethanol) and purified FabH (50 µg) was added which resulted in a 300 µL reaction mixture. Cerulenin was added to inhibit any undesirable chain elongation. The enzyme mixture was incubated 37 °C for 2.5 h. The mixture was centrifuged at room temperature for 5 min at 8000 rpm. The supernatant was separated and tested for production of 3-hydroxybutyryl-CoA using HPLC.

Cascade reaction with AdhE: The activities of FabH/ACP and AdhE were coupled together in presence of NADH in a cascade reaction. The quantity of AdhE and NADH to be added was optimized for the reaction. 100 µL of the reaction mixture was used for the assay, to which 5 mM of NADH solution and 25 µL of AdhE purified enzyme (200 µg) was added. The reaction mixture was mixed thoroughly and incubated for 2.5 hours at 37 °C. The enzymatic mixture was centrifuged for 5 min at 8000 rpm. The supernatant was analysed by HPLC and GC-MS. The production of 1,3-BDO was confirmed using HPLC.

Analysis of 3-hydroxybutyryl-CoA, 1,3-BDO and 1,3-BD: 3-Hydroxybutyryl-CoA and 1,3-BDO were analyzed on HPLC. The conditions for the analysis of 3-hydroxybutyryl-CoA includes using a C-18 column and isocratic mobile phase consisting of 100 mM ammonium acetate with 9% methanol and 0.1% formic acid at a flow rate of 1.0 mL min^{-1}. Column temperature was maintained at 25 °C. The retention time for standard 3-hydroxybutyryl-CoA was 10.3 min.

The conditions for the analysis of 1,3-BDO includes using anion exchange column and the mobile phase used was 5 mM sulfuric acid solution at a flow rate of 0.6 mL min^{-1}. Column temperature was maintained at 50 °C. The retention time for standard 1,3-BDO was 18.0 min.

For analysis of 1,3-BD, headspace sample of the sealed vial was withdrawn and injected on the GC system (DB-1 column, 100 x 0.5 mm, ID: 0.25 mm). Parameters for GC analysis were: FID detector temperature: 160 °C, column temperature: 50 °C, gas flow rate: 25 mL min^{-1}. The retention time of standard 1,3-BD was 5.9 min.

Sample preparation for ^{1}H-NMR: The head space aliquot was sampled and bubbled into the $CDCl_3$ solution in NMR tube which was kept at -10 °C. The NMR tube was sealed and recorded instantly using Bruker NMR instrument.

Chemical catalysis reaction and conditions: All reactions were carried out in oven dried glassware or in cylindrical reaction vials with rubber cork. All solvents were purchased from local suppliers and were of laboratory reagent grade. Dry acetone was prepared by standard protocol and was stored under 4 Å molecular sieves. 1H-NMR (400 MHz) spectra was recorded in $CDCl_3$ and chemical shifts are given in part per million (ppm). 1H-NMR spectra are referenced to $CDCl_3$ (δ =7.26 ppm). The multiplicity

is given as, s = singlet, d = doublet, m = multiple and coupling constants J are reported in Hz.

Chemical conversion of 1,3 BDO to 1,3 BD: To 1 g of 1,3-BDO, 85% H_3PO_4 or zeolite NaY or a combination of both H_3PO_4 and zeolite NaY was added and heated. The progress of the reaction was monitored using GC by injecting a small aliquot (0.1 mL) of samples drawn from the headspace of the reaction vessels.

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Keywords: 1,3-Butadiene • Decarboxylative Claisen condensation • Enzymes • Metabolism • Syngas

[1] K. T. Roseno, R. M. B. Alves, R. Giudici, M. Schmal in *Biofuels-State of Development*, (Ed.: K. Biernat), IntechOpen, London, **2018**, pp 273-290.
[2] N. Déparrois, P. Singh, K. G. Burra, A. K. Gupta, *Appl. Energy.* **2019**, *246*, 1.
[3] J. Shen, M. Li, W. Li, S. Lin, Z. Hao, X. Chang, J. Lv, X. Ma, *Sci. Technol.* **2020**, *10*, 4340.
[4] A. Masudi, N. W. C. Jusohac, O. Muraza, *Catal. Sci. Technol.* **2020**, *10*, 1582.
[5] B. Acharya, P. Roy, A. Dutta, *Biofuels* **2014**, *5*, 551.
[6] X. Sun, H. K. Atiyeh, R. L. Huhnke, Tanner, R.S. *Bioresour. Technol*. Rep. **2019**, *7*, 100279.
[7] J. Jack, J. Lo, P. C. Maness, Z. J. Ren, *Biomass Bioenergy* **2019**, *124*, 95.
[8] N. L. Morrow, *Environ Health Perspect.* **1990**, *86*, 7.
[9] E. V. Makshina, M. Dusselier, W. Janssens, J. Degrève, P. A. Jacobs, B. F. Sels, *Chem. Soc. Rev*. **2014**, *43*, 7917.
[10] "1,3 Butadiene Market Forecast, Trend Analysis & Competition Tracking - Global Review 2019 to 2029" can be found under https://www.factmr.com/report/3783/1-3-butadiene-market
[11] Y. Qi, Z. Liu, S. Liu, L. Cui, Q. Dai, J. He, W. Dong, C. Bai, *Catalysts* **2019**, *9*, 97.
[12] G. Kim, C. Lee, B. Yang, J. Chai (LG Corp), U.S. Patent-6753382, **2004**.
[13] C. Brasse, T. Haas, R. Weber, J. Neuroth (Evonik Operations GmbH), U.S. Patent-20030212298, **2003**.
[14] J. Yang, C. T. Zhang, X. J. Yuan, M. Zhang, X. H. Mo, L. L. Tan, L. P.Zhu, W. J. Chen, M. D. B. Yao, Hu, S. Yang, *Microb. Cell Fact*. **2018**, *17*, 194.
[15] L. H. Ederington, C. S. Fernando, S. A. Hoelscher, T. K. Lee, S. C. Linn, *J. Commod.* Mark. **2019**, *13*, 1.
[16] F. Ahmed, A. N. M. Fakhruddin, *Int. J. Environ. Sci. Nat. Res.* **2018**, *11*, 63.
[17] B. Sivaraman, S.S. Badle, V. Rangaswamy, *ChemistrySelect* **2016**, *1*, 4630.
[18] P. S. Pearlman, C. Chen, A. L. Botes, A. V. E. Conradie (Invista North America SARL), U.S. Patent-20160355844, **2016**.
[19] R. E. Osterhout, A. P. Burgard, P. Pharkya, M. J. Burk (Genomatica Inc), U.S. Patent-9556461, **2015**.
[20] M. J. Burk, A. P. Burgard, J. Sun, R. E. Osterhout, P. Pharkya (Genomatica Inc), U.S. Patent-9732361, **2014**.
[21] S. J. Culler, R. J. Haselbeck, H. Nagarajan, I. E. Gouvea, D. J. Koch, M. S. G. Lopes, L. P. Parizzi (Braskem SA , Genomatica Inc), U.S. Patent-20190010479, **2019**.
[22] C. Y. Lai, J. E. Cronan, *J. Biol. Chem*. **2003**, *278*, 51494.
[23] A. Klein, K. Keisers, R. Palkovits, *Appl. Catal.*, A. 2016, 514, 192.
[24] D. E. Bryant, W. L. Kranich, *J. Catal.* **1967**, *8*, 8.
[25] K. A. Tarach, J. Tekla, W. Makowski, U. Filek, K. Mlekodaj, V. Girman, M. Choi, K. Góra-Marek, *Catal. Sci. Technol*. **2015**, *6*, 3568.
[26] H. Duan, Y. Yamada, S. Sato, *Chem. Lett*. **2016**, *45*, 1036.
[27] F. Jing, B. Katryniok, M. Araque, R. Wojcieszak, M. Capron, S. Paul, M. Daturi, J. M. Clacens, F. De Campo, A. Liebens, F. Dumeignilae, Pera-Titus, M. *Catal. Sci. Technol*., **2016**, *6*, 5830.
[28] H. A. M. Duisters, *Ind. Eng. Chem.* Res. **1994**, *33*, 171.
[29] D. Song, *Catalysts* **2018**, *8*, 72.
[30] J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, New York, **1989**.

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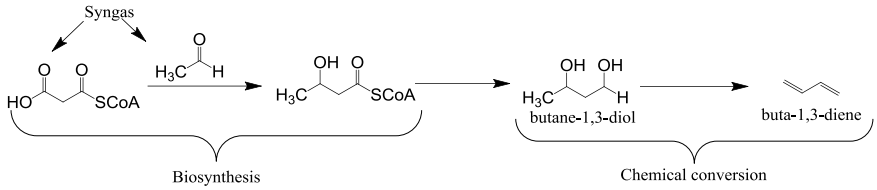

A novel pathway to convert syngas to 1,3-butadiene using a combination of biological and chemical catalysis is proposed. In vitro demonstration of Decarboxylative Claisen Condensation by FabH using unnatural substrates is the highlight of this study. A unique combination of zeolites and inorganic acid facilitated the dehydration of 1,3-butanediol to 1,3-butadiene with 88 % yield in a fixed bed reactor.