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Catalytic Upgrading in Bacteria-Compatible Conditions via a Biocompatible Aldol Condensation

Dylan W. Domaille^{1,*}, Glenn R. Hafenstine¹, Mattias A. Greer¹, Andrew P. Goodwin^{1,2,*}, and Jennifer N. Cha^{1,2,*}

¹Department of Chemical and Biological Engineering, University of Colorado, 3415 Colorado Ave., Boulder, Boulder, CO 80303

²Materials Science and Engineering Program, University of Colorado, 3415 Colorado Ave., Boulder, Boulder, CO 80303

Abstract

Integrating non-enzymatic chemistry with living systems has the potential to greatly expand the types and yields of chemicals that can be sourced from renewable feedstocks. The in situ conversion of microbial metabolites to higher order products will ensure their continuous generation starting from a given cellular reaction mixture. We present here a systematic study of different organocatalysts that enable aldol condensation in biological media under physiological conditions of neutral pH, moderate temperature, and ambient pressure. The relative toxicities of each catalyst were tested against bacteria, and the catalysts were found to provide good yields of homoaldol products in bacterial cultures containing aldehydes. Lastly, we demonstrate that a biocompatible oil can be used to selectively extract the upgraded products, which enables facile isolation and decreases the product toxicity to microbes.

TOC image

Integrating a biocompatible organocatalytic aldol reaction improves microbial cell health by converting short-chain aliphatic aldehydes to insoluble upgraded products.

*To whom correspondence should be addressed to: jennifer.cha@colorado.edu; Dylan.Domaille@colorado.edu; Andrew.Goodwin@colorado.edu.

Supporting Information

IC₅₀ measurements, biocompatibility measurements, ¹H NMR spectra, and *E. coli* catalyst consumption studies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

organocatalysis; microbes; green chemistry; 2-ethylhexenal; β -alanine; glycerol tributyrates

Introduction

One of the grand challenges for green chemistry and engineering is to find an efficient, clean, and cost-effective means of converting raw materials into technologically relevant products. In recent years, advancements in synthetic biology have yielded processes that utilize the machinery of microbes to convert sugars into biofuels,^{1–8} drugs,^{9–12} and industrially relevant chemicals.^{13–17} In these approaches, the desired product is either directly produced by the microbe, or the small molecule metabolites are removed from the media and chemically upgraded in a separate flask.^{18,19} However, the accumulation of the desired product or product intermediates can harm or kill the microbe.^{20–22} In addition, product isolation such as distillation from complex growth media can be energy-intensive.²³ Here, these challenges are addressed simultaneously by integrating microbial production of common C₃–C₆ fermentation products into water-insoluble hydrocarbons using concomitant organocatalytic upgrading in a single-flask process. This process mitigates metabolite toxicity to the microbe, enables facile extraction of the desired product, and greatly expands the overall yield and collection of chemicals that can be sourced from photosynthetically captured carbon.

To develop a one-pot process for upgrading bacterial metabolites, several significant challenges must be addressed: the catalyst must (a) operate in aqueous growth media at physiological temperature and pressure without becoming inactivated by media components;²⁴ (b) exhibit high microbial biocompatibility; (c) perform efficiently at low metabolite concentration; and (d) yield an upgraded product with physical properties that enable low-energy product isolation. These challenges have been elegantly outlined in a recent review.²⁵ Several elegant examples of integrated one-pot enzyme/chemical catalysis processes have been reported,^{26,27} though the study of whole cells in the presence of chemical catalysts has been studied far less.²⁸ However, some work has been performed in this area. Balskus *et al* have merged non-enzymatic catalysis with microbial metabolite

production and demonstrated hydrogenation of exogenously added alkenes in media with hydrogen-producing *E. coli* and biocompatible Pd catalysis.²⁹ Recently, this area has been expanded to include the iron-catalyzed cyclopropanation of microbially produced styrene in a single-flask process³⁰ and the electrocatalytic upgrading of metabolically produced muconic acid to 3-hexenedioic acid in the presence of biogenic impurities.³¹

In consideration of these challenges, we hypothesized that an organocatalyzed aldol reaction would not only function in growth conditions but would also result in a doubling of the carbon content in the product, thereby enabling its separation from the aqueous media. This solvent-driven removal of microbial product would ensure a low aqueous concentration of the intrinsically toxic metabolites. Furthermore, because organocatalysts are commonly derived from amino acids or other biogenic amines,^{32–34} it is likely that the organocatalyst would exhibit good biocompatibility with living cells, which would allow high catalyst loadings and help drive the reaction at low aldehyde concentrations. Finally, in terms of aldehyde production from cells, aliphatic aldehydes should be attainable from genetically engineered bacteria, as these are often penultimate precursors in alcohol biosynthesis. For instance, *Z. mobilis* ferments glucose to acetaldehyde, which is subsequently reduced to ethanol.³⁵ *C. acetobutylicum* ferments glucose to n-butanal in the penultimate step of n-butanol synthesis,³⁶ and engineered pathways to produce n-butanol and isobutanol in *E. coli* also use n-butanal and isobutyraldehyde as intermediates *en route* to the final alcohols.^{1,4} Though extensive work has been performed by synthetic biologists to produce chemicals that can be used directly as fuel, we reasoned that utilizing the C-C bond forming potential of the intermediate aldehydes would greatly expand the breadth of compounds that could be sourced from biomass, and the work shown here establishes the guiding principles to do so.

Here, we show that 3-aminopropionic acid (β -alanine), an inexpensive organocatalyst, exhibits excellent biocompatibility and catalyzes the conversion of n-aliphatic aldehydes to their corresponding homoaldol products at physiological conditions (pH 7.4, 37°C) in good yield and with high selectivity. In a biphasic system with glycerol tributyrate (GT, 10% v/v), catalytic upgrading of C₃–C₆ aldehydes to their C₆–C₁₂ products occurs in the aqueous phase, and the increased lipophilicity of the C₆–C₁₂ product allows for selective accumulation in the GT phase, effectively sequestering the final condensation products from the microbe to help maintain cell viability.

Results

In initial studies, amino acids and biogenic amines were screened for homoaldol condensation activity of n-butanal to 2-ethylhexenal (2-EH) in conditions compatible with *E. coli* growth and maintenance (Table 1). To start, we chose n-butanal as the reactant, as it has been shown that it should be possible to produce this from *C. acetobutylicum*²⁶ or in genetically engineered *E. coli*.¹ Furthermore, the condensation product 2-EH is highly insoluble in water and can therefore be easily extracted into an oil phase. Using a model system of simulated physiological condition (pH 7.4 PBS, 25 mol% catalyst), we observed through ¹H NMR and mass analysis that glycine could convert n-butanal (500 mM) to 2-EH in high purity but in a low yield of 26% after 24 h whereas β -alanine produced higher yields (51%), and 4-aminobutyric acid, 5-aminovaleric acid, and 6-aminocaproic acid all provided

similar yields of 2-EH (*ca.* 40%). Similar to previous reports, lysine showed excellent activity and was capable of converting 59% of n-butanal to 2-ethylhexenal at physiological temperature and pH at this relatively high concentration of n-butanal.³⁷ In each case, NMR analyses showed that only n-butanal and 2-ethylhexenal were observed, indicating minimal side product formation and high product selectivity (Figure S1). In addition to yields, turnover frequencies (TOF) were also determined. As expected, lysine exhibited the highest TOF owing to its lower loading and higher catalytic activity (Table S1).

Because catalysis must occur in the presence of microbes, we next sought to identify the toxicological profile of each candidate catalyst against a model *E. coli* strain, FDA strain Seattle 1946, which is a common host cell for microbial fuel production. Not only would a potentially toxic organocatalyst limit catalyst loading, but toxic effects would also slow the cellular production of metabolites such as n-butanal and therefore decrease yields of target products. To run the cell toxicity assays, we examined the effect of different organocatalyst concentrations on the growth of *E. coli* cultures (Figure S2, Supporting Information). Catalyst concentrations from 0–3.2 M were prepared in minimal media from a 3.2 M stock solution. An aliquot of *E. coli* stock in media (50 μ L of a 1.0 OD stock) was added to the catalyst solution (950 μ L), and the reactions were shaken at 240 RPM at 37°C for 3 h, at which point the reactions were immediately put on ice and the OD₆₀₀ was measured to determine cell density. These studies showed that because lysine was relatively toxic to cells (IC₅₀ ~ 40 mM; Figure 1), this molecule could not be implemented in high enough concentration to be an efficient catalyst. However, in comparison, glycine, 5-aminovaleric acid, and proline showed more moderate toxicity, with measured IC₅₀ values of 170 mM, 350 mM, and 380 mM, respectively (Figure S2). Finally, β -alanine, γ -aminobutyric acid (GABA), and 6-aminocaproic acid all showed excellent biocompatibility (IC₅₀ > 500 mM; Figure S2). Next, to normalize catalytic efficiency with biocompatibility, each organocatalyst was added at its respective IC₅₀ concentration to media in the presence of 70 mM n-butanal (Table 2). This concentration of n-butanal was chosen to match the typical amounts of product that can be generated from engineered microbes.¹ We found that the most biocompatible catalysts provided the highest conversion. Thus, while on a per mol basis lysine is the most efficient catalyst, β -alanine, GABA, and 6-aminocaproic acid all showed higher yields of 2-EH in biocompatible conditions, thus supporting the need to discover efficient catalysts that are well tolerated by cells. Finally, in order to determine if organocatalysts were taken up by cells, 550 mM β -alanine was added to *E. coli* for 24 hours. The cells were removed by centrifugation, and the concentration of β -alanine was determined by ¹H NMR spectroscopy against an internal standard (Figure S3). Triplicate runs revealed near-zero uptake of the β -alanine catalyst by the cells. These results therefore not only support the high biocompatibility of β -alanine but also indicate that these catalysts should be stable to extended incubation with limited catabolism or anabolism by the cells.

Using β -alanine as an efficient, biocompatible catalyst, we then next examined the substrate scope of homoaldol condensation. To do this, 70 mM solutions of n-propanal, n-pentanal, or n-hexanal in minimal media containing 550 mM β -alanine were agitated at room temperature for 24 h followed by isolation of the products via liquid-liquid extraction (Table 2, entries 8–10), and determination of purity by ¹H NMR. The yield of homoaldol product depended on the structure of the initial substrate: propionaldehyde was converted to 2-

methylpentenal and isolated in modest yield (40%), hexanal was converted to 2-butyloctenal in slightly higher yield (57%), and valeraldehyde was converted to its corresponding C₁₀ homoaldol product, 2-propylheptenal, in good yield (80%). The modest yield from propionaldehyde was most likely due to its high volatility (bp 46°C), while the yield of 2-butyloctenal was likely limited by the low solubility of hexanal in aqueous media.

Next, in order to demonstrate the effect of simultaneous catalytic upgrading and extraction on improving cell viability, we incorporated a biocompatible oil phase, glycerol tributyratate (GT) to extract the hydrophobic aldol products as they formed. For this, *E. coli* was incubated with 70 mM n-butanal, 550 mM β -alanine, 10 % v/v GT, or combinations of these. First, as expected, the model product 2-EH was found to be highly toxic to *E. coli* (IC₅₀ = 2.5 mM) (Figure S4). However, incorporation of a 10% v/v GT phase increased the IC₅₀ more than 10-fold (Figure S5). The GT phase itself was also well tolerated by *E. coli* (Table 3, entry 2). Next, we again simulated the production of microbial n-butanal by spiking the cell media with 17.5 mM n-butanal every 12 h over the course of 2 d. Without organocatalyst or GT, continuous introduction and accumulation of n-butanal in the media led to low cell viability (Table 3, entry 3). Introduction of β -alanine to n-butanal without GT led to the complete absence of viable cells owing to the high toxicity of 2-ethylhexenal, which was formed in 62% yield (Table 3, entry 4). However, if both β -alanine and 10% v/v GT were included with n-butanal, the toxicity decreased about 40-fold relative to n-butanal alone. Thus n-butanal was both upgraded into its more lipophilic homoaldol 2-EH product and effectively sequestered away from the bacteria, greatly improving cell health while returning a good yield (70%) of upgraded 2-EH product.

Conclusion

In conclusion, we have shown that inexpensive organocatalysts can carry out aldol condensation reactions in aqueous media at mild temperatures in conditions that are compatible with *E. coli* growth and maintenance. The high biocompatibility and low cost of the β -alanine catalyst (*ca.* <\$0.05 / g from commercial sources) allows for high catalyst loading and efficient reactions at low metabolite concentration. The upgrading reactions also allows for removal of toxic metabolites for improved bacterial viability. Finally, inclusion of a biocompatible oil phase to the reaction mixture can sequester any toxic upgraded products and keep them away from the cellular milieu, thereby improving cell viability. Future studies will focus on engineering bacteria to produce a range of aliphatic aldehydes that can be converted into industrially useful chemicals from renewable feedstocks; other future studies will apply the principles of this work for finding biocompatible catalysts for other reactions relevant to metabolite conversion.

Experimental

General information

Unless otherwise noted, all chemicals and solvents were of analytical grade and used as received from commercial sources. Butyraldehyde, β -alanine, and glycine were from Sigma-Aldrich. Hexanal was supplied by Chem Impex. Propionaldehyde and 6-aminocaproic acid were supplied by TCI. Proline, 5-aminovaleric acid, and lysine were supplied by Acros.

Glycerol tributyrate was supplied by Alfa Aesar, and 4-aminobutyric acid was supplied by Oakwood Chemicals. Water (dd-H₂O) used in biological procedures or as a reaction solvent was deionized using a Milli-Q Advantage A-10 water purification system (MilliPore, USA). ¹H NMR spectra were acquired with a 400 MHz Bruker AV-III spectrometer with a Sample Xpress Automatic Sample Changer. Spectra acquired in CDCl₃ were referenced to residual CHCl₃ (7.27 ppm). Spectra were analyzed with MestreNova 8.1.4. UV-Vis spectra were acquired on a DU 730 spectrophotometer (Beckman Coulter, USA) with quartz cuvettes. Centrifugations were carried out in an X-22R benchtop centrifuge (Beckman Coulter, USA). *E. coli* (ATCC 25922) was used as a cell model for establishing biocompatibility of reaction conditions.

Catalyst screen in minimal media

Solutions containing n-butanal (500 mM) and catalyst (125 mM) were prepared in minimal media. The total reaction volume was 50 mL. The reactions were agitated for 24 h at room temperature, at which point, each reaction was extracted with CH₂Cl₂ (3 × 15 mL). The organic phase was dried over MgSO₄, and the solvent was removed by rotary evaporation at 25°C to obtain the mass of the product, which consisted of unreacted aldehyde and its corresponding homoaldol product. The mass of the product was measured, and the composition of the sample was determined by ¹H NMR.

IC₅₀ measurements

Catalysts—Stock solutions of each catalyst (3200 mM) in minimal media were used to generate working catalyst concentrations of 0, 3.2, 10, 32, 100, 320, 1000, and 3200 mM. An aliquot of *E. coli* stock in media (50 µL of a 1.0 OD solution) was added to the catalyst solution (950 µL), and the reactions were shaken at 240 RPM at 37°C for 3 h, at which point the reactions were immediately put on ice, and the OD₆₀₀ was measured. Each reaction was prepared in triplicate. Reported values represent the mean value of three reactions, and the error bars represent standard deviation. Data were fitted to a four parameter logistic equation to extract IC₅₀ values.

2-ethylhexenal—2-ethylhexenal (2-EH) was prepared according to a previously published procedure.³⁷ A solution of minimal media (950 µL) was spiked with 2-EH to make final concentrations of 0, 0.16, 0.25, 0.40, 0.61, 1.0, 1.6, 2.5, 4.0, 6.3, 10, and 15.8 mM. An aliquot of *E. coli* stock (50 µL of a 1.0 OD culture) was added, and the reactions were shaken at 240 RPM for 4.5 h at 37°C, at which point the OD₆₀₀ values were measured. Data points represent the mean value of three independent values. Error bars represent standard deviation.

Catalyst efficiency at IC₅₀ concentrations

Stock solutions of each catalyst at its IC₅₀ concentration and n-butanal (70 mM) were prepared in minimal media. The total reaction volume was 50 mL. The reactions were agitated for 24 h at room temperature, at which point, each reaction was extracted with CH₂Cl₂ (3 × 15 mL). The organic phase was dried over MgSO₄, and the solvent was removed by rotary evaporation at 25°C to obtain the mass of the product, which consisted of

unreacted aldehyde and its corresponding homoaldol product. The mass of the product was measured, and the composition of the sample was determined by ^1H NMR.

Biocompatibility Measurements

Solutions were prepared to test cell health in combinations of reaction components. The total reaction volume was 10 mL. β -alanine (550 mM), glycerol tributyrate (10% v/v), and *E. coli* stock (500 μL of a 1.0 OD culture) were mixed with minimal media. n-Butanal was added in increments of 17.5 mM every 12 h until a final concentration of 70 mM was reached. The reactions were agitated for 48 h at room temperature, at which point aliquots (50 μL) for gel plate assays were removed. The reaction was extracted with CH_2Cl_2 , the organic phase was concentrated, and the mass was measured. Analysis by ^1H NMR enabled calculation of the relative composition of the mixture, which consisted of unreacted aldehyde, aldol condensation product, glycerol tributyrate, and trace CH_2Cl_2 .

Plate Assay for Cell Viability

Because solutions with glycerol tributyrate (GT) are capable of scattering light and conflating OD_{600} measurements, we used plate assays to calculate the number of colony forming units (CFU) per mL in experiments containing GT. Serial dilutions of each reaction (10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6}) were prepared in minimal media, and 50 μL of each sample was spread evenly with a sterile loop on an LB plate. The plates were incubated overnight at 37°C , and the distinct colonies were counted to obtain CFU/mL values.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bond-Watts BB, Bellerose RJ, Chang MCY. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. *Nat Chem Biol.* 2011; 7(4):222–227. [PubMed: 21358636]
2. Atsumi S, Higashide W, Liao JC. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat Biotech.* 2009; 27(12):1177–1180.
3. Baez A, Cho K-M, Liao J. High-flux isobutanol production using engineered *Escherichia coli*: a bioreactor study with in situ product removal. *Appl Microbiol Biotechnol.* 2011; 90(5):1681–1690. [PubMed: 21547458]
4. Higashide W, Li Y, Yang Y, Liao JC. Metabolic Engineering of *Clostridium cellulolyticum* for Production of Isobutanol from Cellulose. *Appl Environ Microbiol.* 2011; 77(8):2727–2733. [PubMed: 21378054]
5. Georgianna DR, Mayfield SP. Exploiting diversity and synthetic biology for the production of algal biofuels. *Nature.* 2012; 488(7411):329–335. [PubMed: 22895338]
6. Rabinovitch-Deere CA, Oliver JWK, Rodriguez GM, Atsumi S. Synthetic Biology and Metabolic Engineering Approaches To Produce Biofuels. *Chem Rev.* 2013; 113(7):4611–4632. [PubMed: 23488968]

7. Zheng Y, Liu Q, Li L, Qin W, Yang J, Zhang H, Jiang X, Cheng T, Liu W, Xu X, et al. Metabolic engineering of *Escherichia coli* for high-specificity production of isoprenol and prenol as next generation of biofuels. *Biotechnol Biofuels*. 2013; 6(1):57. [PubMed: 23618128]
8. Kallio P, Pásztor A, Thiel K, Akhtar MK, Jones PR. An engineered pathway for the biosynthesis of renewable propane. *Nat Commun*. 2014; 5
9. Martin VJJ, Pitera DJ, Withers ST, Newman JD, Keasling JD. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. *Nat Biotech*. 2003; 21(7):796–802.
10. Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J, et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*. 2006; 440(7086):940–943. [PubMed: 16612385]
11. Neumann H, Neumann-Staubitz P. Synthetic biology approaches in drug discovery and pharmaceutical biotechnology. *Appl Microbiol Biotechnol*. 2010; 87(1):75–86. [PubMed: 20396881]
12. Ajikumar PK, Xiao W-H, Tyo KEJ, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G. Isoprenoid Pathway Optimization for Taxol Precursor Overproduction in *Escherichia coli*. *Science*. 2010; 330(6000):70–74. [PubMed: 20929806]
13. Robertson DE, Jacobson SA, Morgan F, Berry D, Church GM, Afeyan NB. A new dawn for industrial photosynthesis. *Photosynth Res*. 2011; 107(3):269–277. [PubMed: 21318462]
14. Zheng Y-N, Li L-L, Liu Q, Yang J-M, Wang X-W, Liu W, Xu X, Liu H, Zhao G, Xian M. Optimization of fatty alcohol biosynthesis pathway for selectively enhanced production of C12/14 and C16/18 fatty alcohols in engineered *Escherichia coli*. *Microb Cell Factories*. 2012; 11(1):65.
15. Deneyer A, Renders T, Van Aelst J, Van den Bosch S, Gabriëls D, Sels BF. Alkane production from biomass: chemo-, bio- and integrated catalytic approaches. *Curr Opin Chem Biol*. 2015; 29:40–48. [PubMed: 26360875]
16. Nikolau BJ, Perera MADN, Brachova L, Shanks B. Platform biochemicals for a biorenewable chemical industry. *Plant J*. 2008; 54(4):536–545. [PubMed: 18476861]
17. Chia M, Schwartz TJ, Shanks BH, Dumesic JA. Triacetic acid lactone as a potential biorenewable platform chemical. *Green Chem*. 2012; 14(7):1850–1853.
18. Anbarasan P, Baer ZC, Sreekumar S, Gross E, Binder JB, Blanch HW, Clark DS, Toste FD. Integration of chemical catalysis with extractive fermentation to produce fuels. *Nature*. 2012; 491(7423):235–239. [PubMed: 23135469]
19. Shanks BH. Unleashing Biocatalysis/Chemical Catalysis Synergies for Efficient Biomass Conversion. *ACS Chem Biol*. 2007; 2(8):533–535. [PubMed: 17708670]
20. Dahl RH, Zhang F, Alonso-Gutierrez J, Baidoo E, Batth TS, Redding-Johanson AM, Petzold CJ, Mukhopadhyay A, Lee TS, Adams PD, et al. Engineering dynamic pathway regulation using stress-response promoters. *Nat Biotech*. 2013; 31(11):1039–1046.
21. Xu Y, Chu H, Gao C, Tao F, Zhou Z, Li K, Li L, Ma C, Xu P. Systematic metabolic engineering of *Escherichia coli* for high-yield production of fuel bio-chemical 2,3-butanediol. *Metab Eng*. 2014; 23(0):22–33. [PubMed: 24525331]
22. Kunjapur AM, Prather KLJ. Microbial engineering for aldehyde synthesis. *Appl Environ Microbiol*. 2015
23. Bruggink A, Schoevaart R, Kieboom T. Concepts of Nature in Organic Synthesis: Cascade Catalysis and Multistep Conversions in Concert. *Org Process Res Dev*. 2003; 7(5):622–640.
24. Schwartz TJ, Johnson RL, Cardenas J, Okerlund A, Da Silva NA, Schmidt-Rohr K, Dumesic JA. Engineering Catalyst Microenvironments for Metal-Catalyzed Hydrogenation of Biologically Derived Platform Chemicals. *Angew Chem Int Ed*. 2014; 53(47):12718–12722.
25. Wallace S, Schultz EE, Balskus EP. Using non-enzymatic chemistry to influence microbial metabolism. *Curr Opin Chem Biol*. 2015; 25(0):71–79. [PubMed: 25579453]
26. Vennestrøm PNR, Christensen CH, Pedersen S, Grunwaldt J-D, Woodley JM. Next-Generation Catalysis for Renewables: Combining Enzymatic with Inorganic Heterogeneous Catalysis for Bulk Chemical Production. *ChemCatChem*. 2010; 2(3):249–258.
27. Schwartz TJ, O'Neill BJ, Shanks BH, Dumesic JA. Bridging the Chemical and Biological Catalysis Gap: Challenges and Outlooks for Producing Sustainable Chemicals. *ACS Catal*. 2014; 4(6):2060–2069.

28. Marr AC, Liu S. Combining bio- and chemo-catalysis: from enzymes to cells, from petroleum to biomass. *Trends Biotechnol.* 2011; 29(5):199–204. [PubMed: 21324540]
29. Sirasani G, Tong L, Balskus EP. A Biocompatible Alkene Hydrogenation Merges Organic Synthesis with Microbial Metabolism. *Angew Chem Int Ed.* 2014; 53(30):7785–7788.
30. Wallace S, Balskus EP. Interfacing Microbial Styrene Production with a Biocompatible Cyclopropanation Reaction. *Angew Chem Int Ed.* 2015; 54(24):7106–7109.
31. Suastegui M, Matthiesen JE, Carraher JM, Hernandez N, Rodriguez Quiroz N, Okerlund A, Cochran EW, Shao Z, Tessonnier J-P. Combining Metabolic Engineering and Electrocatalysis: Application to the Production of Polyamides from Sugar. *Angew Chem Int Ed.* 2016; 55(7):2368–2373.
32. Xu LW, Lu Y. Primary amino acids: privileged catalysts in enantioselective organocatalysis. *Org Biomol Chem.* 2008; 6(12):2047–2053. [PubMed: 18528563]
33. Scheffler U, Mahrwald R. Recent Advances in Organocatalytic Methods for Asymmetric C•C Bond Formation. *Chem – Eur J.* 2013; 19(43):14346–14396. [PubMed: 24115407]
34. Meninno S, Lattanzi A. Asymmetric organocatalysis mediated by [small alpha],[small alpha]-l-diaryl prolinols: recent advances. *Chem Commun.* 2013; 49(37):3821–3832.
35. Wecker MSA, Zall RR. Production of Acetaldehyde by *Zymomonas mobilis*. *Appl Environ Microbiol.* 1987; 53(12):2815–2820. [PubMed: 16347497]
36. Rogers P, Palosaari N. *Clostridium acetobutylicum* Mutants That Produce Butyraldehyde and Altered Quantities of Solvents. *Appl Environ Microbiol.* 1987; 53(12):2761–2766. [PubMed: 16347493]
37. Watanabe Y, Sawada K, Hayashi M. A green method for the self-aldol condensation of aldehydes using lysine. *Green Chem.* 2010; 12(3):384–386.

Figure 1.
Representative IC_{50} plots for the organocatalysts studied.

Table 1Screen for biocompatible organocatalysts in aqueous buffer at physiological growth conditions^[a]

Entry ^[a]	Organocatalyst	Isolated yield [%]
1	none	<1
2	glycine	26
3	β-alanine	51
4	γ-aminobutyric acid	40
5	5-aminovaleric acid	39
6	6-aminocaproic acid	42
7	lysine	59
8	DL-proline	23

^[a] Reactions were performed in phosphate buffer (1 M) with n-butanal (500 mM) and the indicated organocatalyst (125 mM) in 50 mL conical tubes at 37°C for 24 h. All yields represent isolated yields of 2-ethylhexenal after liquid-liquid extraction.

Table 2Screen for biocompatible organocatalysts in aqueous buffer at physiological growth conditions.^[a]

Entry	Aldehyde	Organocatalyst	IC ₅₀ [mM]	Yield [%]
1	n-butanal	glycine	170	46
2	n-butanal	β-alanine	550	71
3	n-butanal	γ-aminobutyric acid	540	62
4	n-butanal	5-aminovaleric acid	350	61
5	n-butanal	6-aminocaproic acid	540	62
6	n-butanal	lysine	40	58
7	n-butanal	DL-proline	380	8
8	n-butanal	none	n.a.	0
9	n-propanal	β-alanine	550	40
10	n-pentanal	β-alanine	550	80
11	n-hexanal	β-alanine	550	57

^[a] Reactions were performed in minimal media with the indicated aldehyde (70 mM) and the indicated organocatalyst loaded at its IC₅₀ concentration in 50 mL conical tubes at 37°C for 24 h. All yields represent isolated yields of the homoaldol condensation product after liquid-liquid extraction

Table 3

Entry	Extract.	Organocatalyst ^[b]	RCHO ^[c]	Cell health [CFU/ml $\times 10^8$] ^[d]	Yield 2-EH [%] ^[e]
1	none	none	none	8.4	n.a.
2	GT	none	none	5.6	n.a.
3	none	none	n-butanol	0.02	n.a.
4	none	β -alanine	none	7.4	n.a.
5	none	β -alanine	n-butanol	0	62
6	GT	β -alanine	n-butanol	0.8	70

[a] Reactions were performed in the presence of *E. coli* in minimal media for 2 d at 24.

[b] Reaction carried out in the presence of 400 mM organocatalyst.

[c] n-butanal was added portion wise (17.5 every 12 h) over the course of 48 h.

[d] Cell viability was calculated from a serial dilution plate assay.

$[e]$ Yield was calculated by ^1H NMR.