Biorefinery

International Edition: DOI: 10.1002/anie.201509653
German Edition: DOI: 10.1002/ange.201509653

Combining Metabolic Engineering and Electrocatalysis: Application to the Production of Polyamides from Sugar

Miguel Suastegui[+], John E. Matthiesen[+], Jack M. Carraher, Nacu Hernandez, Natalia Rodriguez Quiroz, Adam Okerlund, Eric W. Cochran, Zengyi Shao, and Jean-Philippe Tessonnier**

Abstract: *Biorefineries aim to convert biomass into a spectrum of products ranging from biofuels to specialty chemicals. To achieve economically sustainable conversion, it is crucial to streamline the catalytic and downstream processing steps. In this work, a route that combines bio- and electrocatalysis to convert glucose into bio-based unsaturated nylon-6,6 is reported. An engineered strain of Saccharomyces cerevisiae was used as the initial biocatalyst for the conversion of glucose into muconic acid, with the highest reported muconic acid titer of 559.5 mg L^{-1} in yeast. Without any separation, muconic acid was further electrocatalytically hydrogenated to 3-hexenedioic acid in 94% yield despite the presence of biogenic impurities. Bio-based unsaturated nylon-6,6 (unsaturated polyamide-6,6) was finally obtained by polymerization of 3-hexenedioic acid with hexamethylenediamine.*

Biomass has emerged as an alternative feedstock to petroleum to render the chemical industry more sustainable and alleviate the concerns associated with fossil resources. The transition from fossil to renewable feedstocks is also expected to revitalize the chemical industry by providing building blocks with new functionalities.[1] Since the U.S. Department of Energy's report on top value-added chemicals from biomass,[2] extensive research has been carried out to establish biological, chemical, or hybrid pathways for converting cellulosic sugars.[3] Over the past few years, it has become evident that building-block diversification requires the combination of biological and chemical transformations,[3b,d,4] that is, biomass is first biologically converted by genetically engineered microbes into platform molecules that are further diversified by chemical catalysis. However, previous attempts to combine chemical and biological processes have led to low conversion rates owing to catalyst deactivation by residual biogenic impurities.[5] The ideal biorefinery pipelines, from biomass to the final products, are currently disrupted by a gap between biological conversion and chemical diversification. We herein report a strategy to bridge this gap with a hybrid fermentation and electrocatalytic process. We illustrate this concept with the conversion of glucose into unsaturated polyamide-6,6 (UPA-6,6). The process entails the fermentation of glucose to muconic acid (MA) followed by electrocatalytic hydrogenation (ECH) to 3-hexenedioic acid (HDA), a monomer that has not been synthesized in high yield through conventional catalytic routes,[6] and subsequent polycondensation with 1,6-hexamethylenediamine (HMDA) to yield the desired UPA-6,6 (Figure 1). The synthesis pipeline developed in this study is based on the utilization of a metabolically engineered yeast and substitution of conventional high-pressure hydrogenation

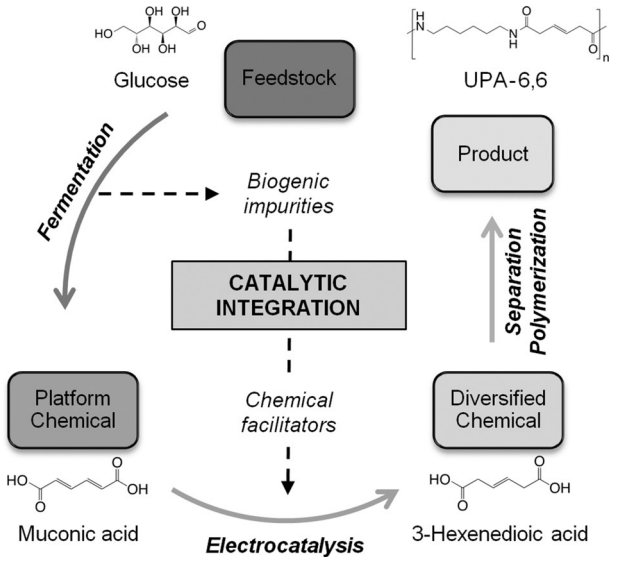

Figure 1. Hybrid conversion of glucose into UPA-6,6. The integration of the catalytic steps was enabled by the compatibility of the process parameters. Replacing conventional high-pressure hydrogenation by direct ECH promoted a seamless flow between the processes, allowing the use of the broth water, salts, and impurities as electrolyte and hydrogen source.

[*] M. Suastegui,[+] J. E. Matthiesen,[+] Dr. J. M. Carraher, Dr. N. Hernandez, Prof. Dr. E. W. Cochran, Prof. Dr. Z. Shao, Prof. Dr. J.-P. Tessonnier
Department of Chemical and Biological Engineering
Iowa State University
Ames, IA 50011 (USA)
E-mail: zyshao@iastate.edu
tesso@iastate.edu
M. Suastegui,[+] J. E. Matthiesen,[+] Dr. J. M. Carraher, N. Rodriguez Quiroz, Dr. A. Okerlund, Prof. Dr. Z. Shao, Prof. Dr. J.-P. Tessonnier
NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
Ames, IA 50011 (USA)
J. E. Matthiesen,[+] Prof. Dr. J.-P. Tessonnier
US Department of Energy Ames Laboratory
Ames, IA 50011 (USA)
[+] These authors contributed equally to this work.

Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/anie.201509653.

by direct ECH without separation using the broth water, salts, and impurities as electrolyte and hydrogen source. Furthermore, we demonstrate that a biobased polymer can be produced according to this combined bio- and electrocatalytic process.

Muconic acid is the unsaturated synthetic precursor of adipic acid and terephthalic acid, which are the monomers of nylon and polyethylene terephthalate (PET), with a total market value greater than $22 billion.[7] The traditional benzene-based synthetic routes for adipic acid and terephthalic acid are environmentally unfriendly,[8] warranting the need for a sustainable and green production platform. For large-scale fermentation, yeast is the preferred microbial host in industry owing to its unique economic advantages, such as the greater ease in maintaining phage-free culture conditions and the sale of biomass byproducts as animal feeds.[9] Two previous reports showed the heterologous production of MA in *S. cerevisiae* with titers of 1.56 mg L^{-1} [10] and 141 mg L^{-1}.[11] The low production was caused by a combination of low precursor availability, active competing pathway(s), and the presence of rate-limiting enzyme(s). To address these individual issues, we performed flux balance analysis (FBA) to obtain a list of target genes for genetic manipulations (Supporting Information, Figure S1). Figure 2 depicts the metabolic pathway with the key manipulations for enhancing MA production. The details of strain construction in this work are listed in Tables S1–S3.

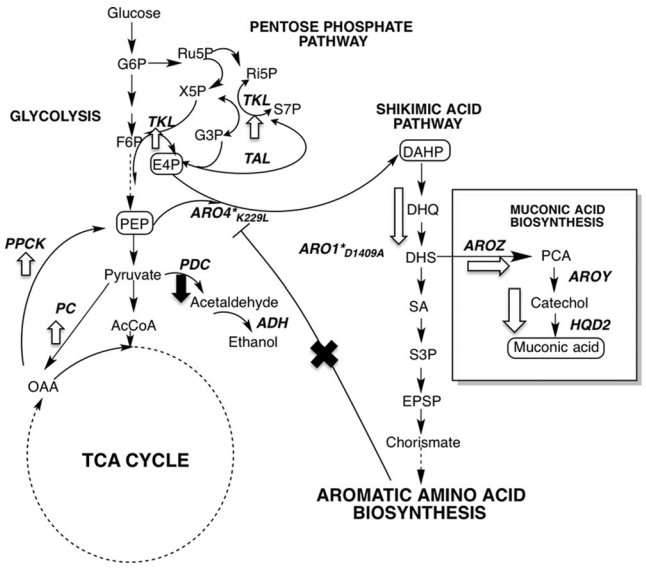

Figure 2. Metabolic engineering rationale for the overproduction of MA in *S. cerevisiae*. Three main strategies were studied: removing the feedback inhibition by aromatic amino acids, increasing the pool of the precursors PEP and E4P, and increasing the pull of carbon into the shikimic acid pathway. Metabolites: G6P: glucose-6-p; F6P: fructose-6-p; Ru5P: ribulose-5-p; Ri5P: ribose-5-p; S7P: sedoheptulose-5-p; X5P: xylulose-5-p; G3P: glyceraldehyde-3-p; AcCoA: acetyl-CoA; PEP: phosphoenolpyruvate; OAA: oxaloacetate; DAHP: 3-deoxy-D-arabinoheptulosonate-7-p; DHQ: 3-dehydroquinate; DHS: 3-dehydroshikimic acid; SA: shikimate; S3P: shikimate-3-p; EPSP: 5-enolpyruvylshikimate-3-p; PCA: protocatechuic acid. White and black arrows represent up- and downregulated reactions, respectively.

The three genes previously characterized in yeast to produce MA from 3-dehydroshikimic acid (DHS), namely *AroZ* from *Podospora anserina*, *AroY* from *Klebsiella pneumoniae*, and *HQD2* from *Candida albicans*,[11] were cloned in a multicopy plasmid. Furthermore, the tyrosine-insensitive DAHP synthase ($ARO4_{K229L}$) was overexpressed to remove feedback inhibition caused by the aromatic amino acids in the growth medium. Following our FBA analysis and previous reports on how to increase the carbon flux into the aromatic amino acid biosynthetic pathway,[11,12] the transketolase gene *(TKL1)* was overexpressed to increase the pool of the precursor erythrose-4-phosphate (E4P). Initial fermentations produced 132 mg L^{-1} MA (strain InvSc1 MA1* in Figure 3a), which is similar to the titer of the previously highest yeast producer MuA12, but the yield was almost doubled.[11] We reasoned that this increase is mostly due to intrinsic differences in the genetic backgrounds of the host strains. Strain dependency in metabolic engineering has also been reported previously.[13] In our case, the strain InvSc1 is a diploid whereas the strain MuA12 derives from the haploid *S. cerevisiae* BY4741.

To further force the carbon flow towards DHS, the flux through the two initial reactions in the aromatic amino acid pathway needs to be increased (Figure 2). As suggested by the FBA (Figure S1), the flux through the DHQ dehydratase had to be increased by a factor of 60 to maximize MA production. In yeast, these two reactions are catalyzed by the pentafunctional ARO1 enzyme,[14] which differs from the stand-alone SA pathway enzymes in bacteria and plants. By sequence alignment of ARO1 with the characterized SA dehydrogenases from various species (Figure S2), it was concluded that the residues K1370 and D1409 could potentially serve as catalytic residues. A panel of plasmids was created to enhance the flux towards DHS and avoid conversion into SA (Figure S3). Overexpression of the mutant $ARO1_{D1409A}$ increased the production of MA to 235 mg L^{-1} (strain InvSc1 MA4), confirming that the residue D1409 is essential for the catalytic activity of the SA dehydrogenase subunit of ARO1. To ensure that no carbon was being diverted to SA, we deleted both copies of *aro1* (InvSc1 MA8 in Figure 3a), but the MA production unexpectedly decreased to 25 mg L^{-1}, and the strain fitness was also affected (Table S4) even when aromatic amino acids were supplemented.

To increase the availability of phosphoenolpyruvate (PEP), we overexpressed *PC* (pyruvate carboxylase) and *PPCK* (phosphoenolpyruvate carboxykinase) in strain InvSc1 MA4, as well as in the strain InvSc1Δ*pdc1* (resulting in the strains InvSc1 MA9 and InvSc1 MA10, respectively). However, the MA titers decreased by around 60% and 74%, respectively (Figure 3a). Recirculation of pyruvate to PEP has been successfully applied in *Escherichia coli* to increase the yield of aromatic compounds.[15] In *S. cerevisiae*, the failure of this strategy might be attributed to the inaccuracy of FBA modeling to predict the compartmentalization fluxes, enzyme kinetics, and the metabolic burden on the cells caused by overexpression of highly regulated genes.

Despite the aforementioned genetic manipulations, the production of MA was still limited by the accumulation of the intermediate protocatechuic acid (PCA). The enzyme PCA

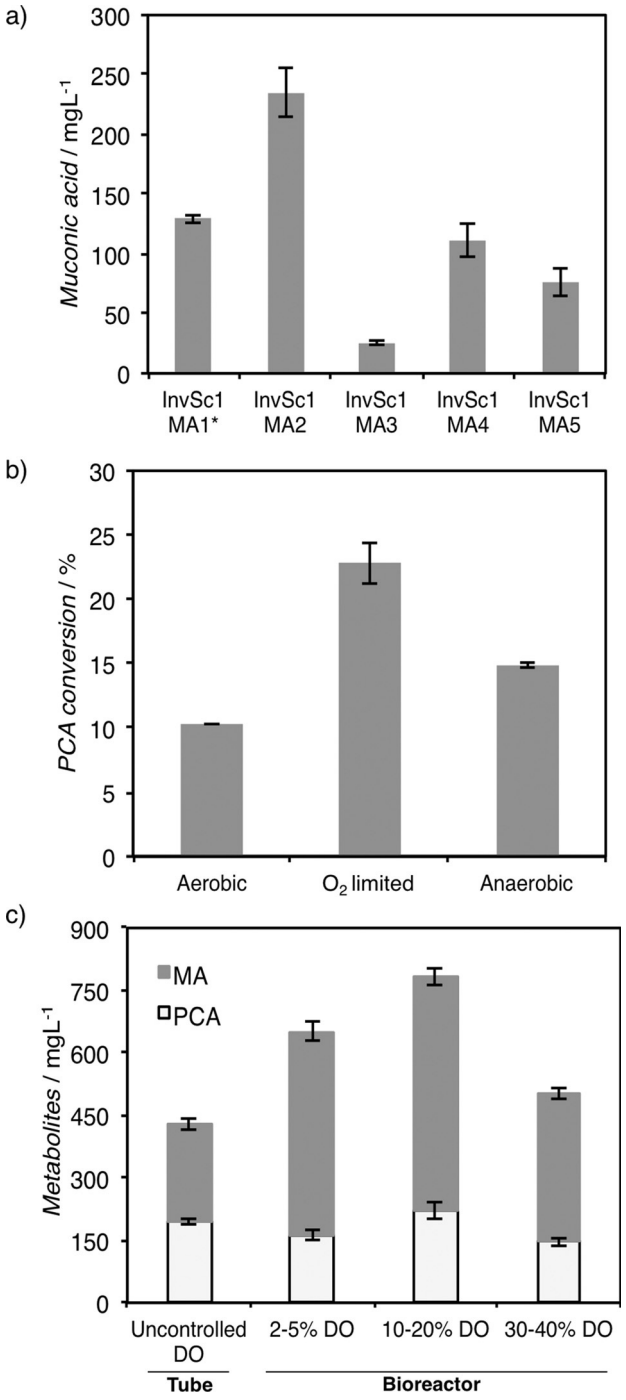

Figure 3. Characterization of MA production in engineered InvSc1 strains. a) MA accumulation in strains grown in glass tubes containing 15 mL of selection media. Maximum MA formation was observed after 96 h of aerobic fermentation. b) PCA decarboxylase activity assay under different oxygen environments. The strain InvSc1 pRS414 aroY was cultured under three different oxygen conditions and spiked with 1 mM PCA after 24 h. The conversions are based on samples collected 18 hours after PCA supplementation. c) Mini-reactor fermentations with the strain InvSc1 MA4 and controlled amounts of dissolved oxygen. The highest MA accumulation was observed when the amount of dissolved oxygen was maintained between 10 to 20% during the first 24 h. After this period, the amount of dissolved oxygen was set to 20% until the end of the fermentation (4 days). *InvSc1 MA1 was grown in 2% glucose, all other strains were grown in 4% glucose.

decarboxylase is known to be oxygen-sensitive;[10,16] we observed that the conversion of PCA into catechol increased by a factor greater than two when the cells were grown in an oxygen-limited culture (Figure 3b). These conditions improved the MA/PCA ratio to 2.5, which is almost five times higher than that of the previously highest MA yeast producer, MuA12.[11] The highest MA titer was 559.5 $mg\,L^{-1}$, representing a fourfold improvement in both titer and yield over strain MuA12 (Figure 3c).

In summary, the MA platform consisted of establishing the heterologous pathway in a diploid yeast strain, overexpressing the novel mutant $ARO1_{D1409A}$, and alleviating the PCA bottleneck with a controlled oxygen environment in the fermentation. The yield of 14 $mg_{MA}\,g_{glucose}^{-1}$ represents the highest value that has been reported for the batch production of aromatic amino acid based metabolites in yeast. Given the strong industrial interest in muconic acid,[17] substantial improvements should be expected in the next several years. Mutagenesis of AroY to remove its oxygen sensitivity[18] and global genetic perturbations[19] coupled with molecular sensor development[20] are potential strategies that will enable yeast to reach the high yields as observed with the *E. coli* platforms.[7b,15b]

The fermentation broth was subsequently hydrogenated in a three-electrode electrochemical cell (Figure 4a). Electrocatalysis was preferred over conventional high-pressure hydrogenation as hydrogen is produced in situ by water splitting, the reaction occurs at ambient temperature and pressure, and the charge on the electrode surface can mitigate poisoning.[21] In this configuration, hydrogen production and MA hydrogenation take place simultaneously at the cathode [Eq. (1) and (2)], enabling a seamless ECH.

$$H^+ + e^- + * \rightarrow H^* \quad (1)$$

$$2\,H^* + C_6H_6O_4 + * \rightarrow C_6H_8O_4 \quad (2)$$

Lead (Pb) was chosen as the catalyst because of its earth abundance, low cost, common use in industrial electrosynthetic applications, metallic state under cathodic potentials, and its stability in the presence of sulfur.[22] The expected resistance to impurities allowed us to significantly simplify the hydrogenation reaction by placing the fermentation broth directly in the electrochemical reactor. The broth contained whole yeast cells, unspent salts, and biogenic impurities arising from cellular metabolism and lysis. The ECH was then allowed to proceed at room temperature and atmospheric pressure for one hour at a potential of −1.5 V vs. Ag/AgCl on a 10 cm^2 lead rod, resulting in 95% MA conversion with 81% selectivity to HDA. To assess the stability of the catalyst in the fermentation medium (in the presence of all potential poisons), five successive one-hour electrocatalytic batch reactions were performed (Figure 4b). Notably, no signs of deactivation were observed, and leaching of the catalyst into the solution was minimal at 6.5 ± 0.4 ppm as determined by elemental analysis.

To further increase the yield of HDA, the effects of pH and applied voltage were investigated independently. A model solution of pure MA dissolved in a potassium sulfate/

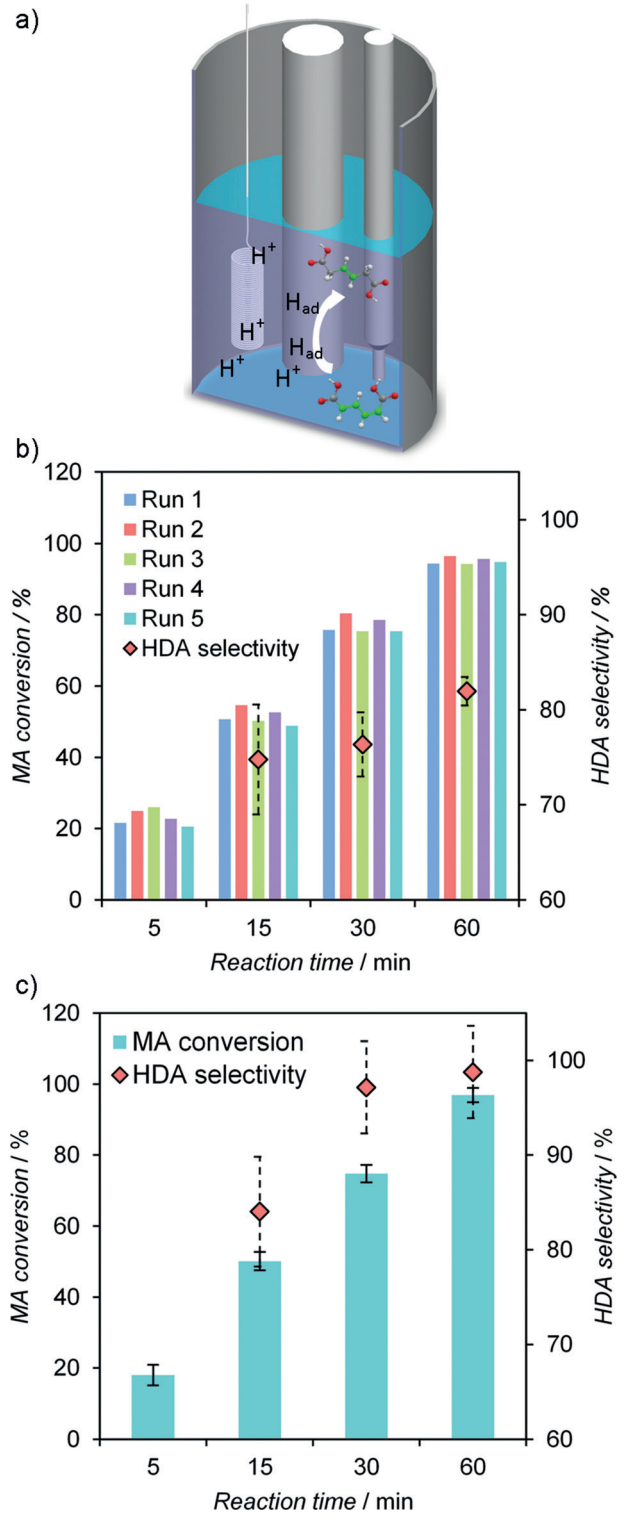

Figure 4. Electrocatalytic hydrogenation of MA to HDA directly in the fermentation broth. The hydrogen necessary for the reaction is generated in situ (H_{ad}) at the surface of the Pb electrode. a) Electrocatalytic single-cell reactor for the conversion of MA into HDA. Carbon gray, hydrogen white, nitrogen blue, oxygen red, C=C bonds green. The reaction was performed at ambient temperature and pressure using a three-electrode electrochemical cell at −1.5 V vs. Ag/AgCl. b) Conversion of MA and average selectivity to the desired product showed no signs of catalyst deactivation when the reaction was repeated five times (runs 1–5). c) MA conversion and HDA selectivity for the ECH (−1.5 V) of the fermentation broth at pH 2.0.

sulfuric acid electrolyte was used to accurately control the ionic strength and to maintain constant ionic conductivity. Acidic conditions favored the selective formation of HDA, especially for reaction times below 30 min (Figure S4). Further ^{1}H NMR analysis of a HDA model solution after ECH revealed that the observed decrease in selectivity as the reaction proceeded was due to the formation of decomposition products through secondary reactions and not due to the formation of additional hydrogenation products, for example, adipic acid (Figure S5). These undesired reactions were enhanced when the pH and/or the applied cathodic voltage were increased (Figure S6). A potential of −1.5 V and a pH of 2.0 offer a compromise between conversion and selectivity.

The conditions optimized with the model solutions were found to also enhance the hydrogenation of the fermentation broth (Figure 4c). Notably, when the pH of the solution was fixed at 2.0, the selectivity towards HDA became 98 ± 4% at 96 ± 2% MA conversion. It is worth noting that the yield achieved for the unpurified broth was actually higher than for the model solution (94% vs. 77%). Whereas catalyst poisoning is a common issue for most of the hydrogenation reactions catalyzed by precious metals,[3d,5,23] it appears that the impurities in the broth were beneficial in our case as they prevented the formation of decomposition products during the ECH.

The reduced decomposition when the fermentation broth is reacted suggests a competitive adsorption process with impurities in solution being sacrificed to minimize potential HDA oxidation. To the best of our knowledge, this is the first time that a biologically produced chemical was hydrogenated in high yield and selectivity directly in the fermentation broth in the presence of diverse impurities.

To demonstrate a full conversion pipeline for the transformation of glucose into a commercially viable product, HDA was separated from the fermentation broth by vacuum evaporation, filtration over activated carbon, and crystallization. High-purity (98%) HDA was obtained in a yield of 67% (Figure S7) and subjected to the final polymerization step. The corresponding saturated nylon-6,6 was synthesized using adipic acid and HMDA in an attempt to compare conventional petrochemical-based nylon-6,6 and bio-based UPA-6,6. The obtained UPA-6,6 consisted of a transparent, partially crystallized material with physical and chemical properties comparable to those of petrochemical nylon (Table 1, Figure 5; see also Table S5, Figure S8–S12). Polymers based on blends of HDA and adipic acid were also synthesized (Figure 5a) to enable different levels of tunability. These HDA-containing nylon materials offer precious grafting sites to tailor the existing nylon grades with desirable properties, for example, crosslinkability, paintability, and flame retardancy.

In conclusion, we have presented a strategy to bridge the gap between biological and chemical catalysis in biorefinery. We have demonstrated its potential by synthesizing a new family of unsaturated polyamides from sugar and anticipate that this strategy will facilitate the incorporation of fermentation and catalytic hydrogenation for a broad range of reactions. Future efforts will be directed towards 1) the scale-up of the hybrid pipeline with a detailed techno-economic

Table 1: The properties of Nylon-6,6 and UPA-6,6.[a]

Property	Nylon-6,6	UPA-6,6
M_n (Da)	17800	12200
PDI	2.0	3.36
T_{melt} [°C]	250	60
Q^* [Å^{-1}]	4.4	4.7
G' [MPa]	–	18.9
G'' [MPa]	–	6.24
G''_c [°C]	–	60

[a] M_n: number average molecular weight; PDI: polydispersity; Q^*: primary diffraction peak; G': storage modulus; G'': loss modulus; G^*_c: crossover modulus.

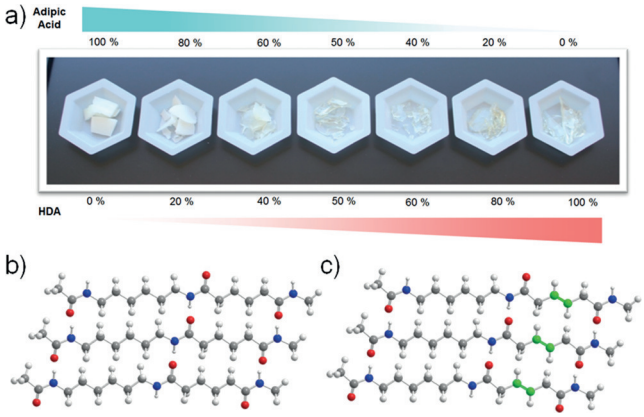

Figure 5. a) Polymer blends of adipic acid and HDA. Percentages are based on the molar ratios of adipic acid and HDA reacted with a 1:1 ratio of HMDA. b, c) Structures of Nylon-6,6 (b) and UPA-6,6 (c), for which petroleum-based adipic acid was substituted with HDA. Carbon gray, hydrogen white, nitrogen blue, oxygen red, C=C bonds green.

analysis to assess the cost efficiency and 2) the integration of the biocatalytic and chemocatalytic reactions into a one-pot process to develop economically and ecologically advantageous synthesis schemes in the context of the water, energy, and food nexus.[4d]

Acknowledgements

We sincerely thank CBiRC for providing the resources that enabled this collaborative work. This material is based upon work supported in part by the National Science Foundation (EEC-0813570 and EPSC-1101284) and the Plant Sciences Institute at Iowa State University. Research at the Ames Laboratory was supported by the U.S. Department of Energy-Laboratory Royalty Revenue (DE-AC02-07CH11358). We thank Dr. Sarah Cady (ISU Chemical Instrumentation Facility) for training and assistance pertaining to the AVIII-600 results included in this publication, Dr. Jieni Lian, Mengguo Yan, Jacob Neff, Jennifer Freeland, Shridharshna Mailachalam, and Andrew Moon for technical assistance, and Taylor Royer for the preparation of Figure 4 A.

Keywords: bio-based polymers · biorefinery · electrocatalysis · metabolic engineering · muconic acid

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 2368–2373
Angew. Chem. **2016**, *128*, 2414–2419

[1] N. Hernández, R. C. Williams, E. W. Cochran, *Org. Biomol. Chem.* **2014**, *12*, 2834.
[2] J. J. Bozell, G. R. Petersen, *Green Chem.* **2010**, *12*, 539.
[3] a) S. Choi, C. W. Song, J. H. Shin, S. Y. Lee, *Metab. Eng.* **2015**, *28*, 223; b) A. Corma, S. Iborra, A. Velty, *Chem. Rev.* **2007**, *107*, 2411; c) J. Becker, C. Wittmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 3328; *Angew. Chem.* **2015**, *127*, 3383; d) T. J. Schwartz, B. J. O'Neill, B. H. Shanks, J. A. Dumesic, *ACS Catal.* **2014**, *4*, 2060.
[4] a) J. G. Linger, D. R. Vardon, M. T. Guarnieri, E. M. Karp, G. B. Hunsinger, M. A. Franden, C. W. Johnson, G. Chupka, T. J. Strathmann, P. T. Pienkos, G. T. Beckham, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12013; b) D. R. Vardon, M. A. Franden, C. W. Johnson, E. M. Karp, M. T. Guarnieri, J. G. Linger, M. J. Salm, T. J. Strathmann, G. T. Beckham, *Energy Environ. Sci.* **2015**, *8*, 617; c) B. J. Nikolau, M. A. D. N. Perera, L. Brachova, B. Shanks, *Plant J.* **2008**, *54*, 536; d) H. Gröger, W. Hummel, *Curr. Opin. Chem. Biol.* **2014**, *19*, 171.
[5] a) Z. Zhang, J. E. Jackson, D. J. Miller, *Bioresour. Technol.* **2008**, *99*, 5873; b) T. J. Schwartz, R. L. Johnson, J. Cardenas, A. Okerlund, N. A. Da Silva, K. Schmidt-Rohr, J. A. Dumesic, *Angew. Chem. Int. Ed.* **2014**, *53*, 12718; *Angew. Chem.* **2014**, *126*, 12932.
[6] J. A. Elvidge, R. P. Linstead, J. F. Smith, *J. Chem. Soc.* **1953**, 708.
[7] a) M. J. Burk, R. E. Osterhout, J. Sun, US 20110124911 A1, **2011**; b) W. Niu, K. M. Draths, J. W. Frost, *Biotechnol. Prog.* **2002**, *18*, 201; c) S. Picataggio, T. Beardslee, US8241879 B2, **2012**.
[8] S. Alini, F. Basile, S. Blasioli, C. Rinaldi, A. Vaccari, *Appl. Catal. B* **2007**, *70*, 323.
[9] a) M.-J. In, D. C. Kim, H. J. Chae, *Biotechnol. Bioprocess Eng.* **2005**, *10*, 85; b) T. W. Jeffries, *Curr. Opin. Biotechnol.* **2006**, *17*, 320; c) J. Nielsen, *Science* **2015**, *349*, 1050; d) J. Nielsen, C. Larsson, A. van Maris, J. Pronk, *Curr. Opin. Biotechnol.* **2013**, *24*, 398.
[10] C. Weber, C. Bruckner, S. Weinreb, C. Lehr, C. Essl, E. Boles, *Appl. Environ. Microbiol.* **2012**, *78*, 8421.
[11] K. A. Curran, J. M. Leavitt, A. S. Karim, H. S. Alper, *Metab. Eng.* **2013**, *15*, 55.
[12] a) M. A. H. Luttik, Z. Vuralhan, E. Suir, G. H. Braus, J. T. Pronk, J. M. Daran, *Metab. Eng.* **2008**, *10*, 141; b) K. Li, M. R. Mikola, K. M. Draths, R. M. Worden, J. W. Frost, *Biotechnol. Bioeng.* **1999**, *64*, 61; c) Y.-Y. Cui, C. Ling, Y.-Y. Zhang, J. Huang, J.-Z. Liu, *Microb. Cell Fact.* **2014**, *13*, 21/1.
[13] a) J. Du, Y. Yuan, T. Si, J. Lian, H. Zhao, *Nucleic Acids Res.* **2012**, *40*, e142; b) A. Kümmel, J. C. Ewald, S. M. Fendt, S. J. Jol, P. Picotti, R. Aebersold, U. Sauer, N. Zamboni, M. Heinemann, *FEMS Yeast Res.* **2010**, *10*, 322.
[14] G. H. Braus, *Microbiol. Rev.* **1991**, *55*, 349.
[15] a) R. Patnaik, J. C. Liao, *Appl. Environ. Microbiol.* **1994**, *60*, 3903; b) H. Zhang, B. Pereira, Z. Li, G. Stephanopoulos, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8266.
[16] Z. He, J. Wiegel, *J. Bacteriol.* **1996**, *178*, 3539.
[17] a) A. A. Horwitz, J. M. Walter, M. G. Schubert, S. H. Kung, K. Hawkins, D. M. Platt, A. D. Hernday, T. Mahatdejkul-Meadows, W. Szeto, S. S. Chandran, J. D. Newman, *Cell Syst.* **2015**, *1*, 88; b) Y. Rogers, W. Gong, S. Dole, R. Sillers, M. Gandhi, J. Pero, WO2013116244 A1, **2013**.
[18] J. A. Stapleton, J. R. Swartz, *PLoS One* **2010**, *5*, e15275.
[19] a) J. E. DiCarlo, A. J. Conley, M. Penttila, J. Jantti, H. H. Wang, G. M. Church, *ACS Synth. Biol.* **2013**, *2*, 741; b) T. Si, Y. Z. Luo, Z. H. Bao, H. M. Zhao, *ACS Synth. Biol.* **2015**, *4*, 283.

[20] a) S. A. Chugani, M. R. Parsek, A. M. Chakrabarty, *J. Bacteriol.* **1998**, *180*, 2367; b) O. C. Ezezika, L. S. Collier-Hyams, H. A. Dale, A. C. Burk, E. L. Neidle, *Appl. Environ. Microbiol.* **2006**, *72*, 1749.
[21] J. M. Chapuzet, A. Lasia, J. Lessard, *Electrocatalytic Hydrogenation of Organic Compounds*, Wiley, Hoboken, **1998**.
[22] a) R. W. Joyner, K. Kishi, M. W. Roberts, *Proc. R. Soc. London Ser. A* **1978**, *358*, 223; b) D. Pletcher, F. Walsh, *Industrial Electrochemistry*, Springer, Amsterdam, **1993**, p. 481.
[23] J. L. Avalos, G. R. Fink, G. Stephanopoulos, *Nat. Biotechnol.* **2013**, *31*, 335.

Received: October 14, 2015
Published online: January 14, 2016

