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

## Abstract

Biorefineries aim to convert biomass to 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. Here we report a route that integrates bio- and chemical catalysis to convert glucose into bio-based unsaturated nylon 6,6. An engineered strain of *Saccharomyces cerevisiae*, with the highest reported muconic acid titer of 559.5 mg L<sup>-1</sup> in yeast, was used as the initial biocatalyst to convert glucose into muconic acid. Without any separation, muconic acid was further electrocatalytically hydrogenated to 3-hexenedioic acid with 94% yield, despite the presence of all the biogenic impurities. Bio-based unsaturated nylon 6,6 (unsaturated polyamide 6,6) was finally obtained by polymerization of 3-hexenedioic acid with hexamethylenediamine, demonstrating the integrated design of bio-based polyamides from glucose.

## Disciplines

Biochemical and Biomolecular Engineering | Chemical Engineering

## Comments

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## Title: Integrating Metabolic Engineering and Electrocatalysis for the Production of Polyamides from Sugar

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**Abstract:** Biorefineries aim to convert biomass to 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. Here we report a route that integrates bio- and chemical catalysis to convert glucose into bio-based unsaturated nylon 6,6. An engineered strain of *Saccharomyces cerevisiae*, with the highest reported muconic acid titer of 559.5 mg L<sup>-1</sup> in yeast, was used as the initial biocatalyst to convert glucose into muconic acid. Without any separation, muconic acid was further electrocatalytically hydrogenated to 3-hexenedioic acid with 94% yield, despite the presence of all the biogenic impurities. Bio-based unsaturated nylon 6,6 (unsaturated polyamide 6,6) was finally obtained by polymerization of 3-hexenedioic acid with hexamethylenediamine, demonstrating the integrated design of bio-based polyamides from glucose.

**One sentence summary:** The integration of biological and electrochemical catalysis enabled the selective production of a polyamide monomer from sugar.

**Main Text:** Biomass emerges as an alternative feedstock to petroleum to grow a more sustainable chemical industry and alleviate the concerns about 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, extensive research has been carried out to establish biological, chemical, or hybrid conversion pathways for cellulosic sugars (2, 3). Over the past few years, it has become evident that the diversification of building blocks requires the combination of biological and chemical transformations (3-7) that is, biomass is first biologically converted using microbes to platform molecules that are further diversified using chemical catalysis. However, previous attempts to implement chemical and biological processes have led to low conversion rates due to catalyst deactivation by the residual biogenic impurities (8, 9). The ideal biorefinery pipelines, from biomass to the final products, are currently disrupted by a gap between biological conversion and chemical diversification. In this work, we report a strategy to bridge this gap by integrating fermentation and electrocatalytic processing. We illustrate this concept with the conversion of glucose to 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), and subsequent polycondensation with 1,6-hexamethylenediamine (HMDA) to yield the desired UPA 6,6 (Fig. 1). The overall integration in this study is ensured through the utilization of a metabolically engineered yeast and substitution of conventional high-pressure hydrogenation by direct ECH without separation using the broth water, salts, and impurities as an electrolyte and hydrogen source. With this integration, we demonstrate that a bio-based polymer can be produced using a combined bio- and electrocatalytic process.

For large-scale fermentation, yeast is the preferred microbial host in industry with unique economic advantages such as the greater ease in maintaining phage-free culture conditions and by selling biomass byproducts as animal feeds (10, 11). Two previous reports showed the heterologous production of MA in *S. cerevisiae* with titers around 1.56 mg L<sup>-1</sup> and 141 mg L<sup>-1</sup> (12, 13). The low production was caused by a combination of low precursor availability, active competing pathway(s), and the existence of rate-limiting enzyme(s). To address these individual issues, we started with flux balance analysis (FBA) to obtain a list of target genes for genetic manipulations (Fig. S1). Figure S2 depicts the metabolic pathway with the key manipulations for enhancing MA production. The details of strain construction in this work are listed in Table S1-S3.

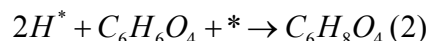
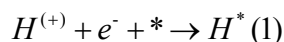
The three genes previously characterized in yeast to produce MA from 3-dehydroshikimic acid (DHS) (13) were cloned in a multiple-copy plasmid. *ARO4<sub>K229L</sub>* (DAHP synthase mutant insensitive to tyrosine feedback inhibition) and *TKL* (transketolase) were also overexpressed. This strategy was implemented to increase the pool of the precursor erythrose-4-phosphate (E4P) according to the FBA analysis and coinciding with previous research (13-16). Initial fermentations produced 132 mg L<sup>-1</sup> MA (strain InvSc1 MA1\* in Fig. 2A), similar to the titer of the previously highest yeast producer MuA12, but the yield was increased almost 2-fold (13). We reasoned that the factors influencing these results are mostly due to the genetic background of the host strain. Du *et al.* (17) showed that tailoring a pathway is impactful to strain performance; in this case the strain InvSc1 is a diploid whereas the strain MuA12 derives from the haploid *S. cerevisiae* BY4741.

In addition to serving as the precursor for MA biosynthesis, DHS is also the key intermediate in aromatic amino acid biosynthesis (Fig. S2). To further force the carbon flow towards DHS, the flux through the two initial reactions in the aromatic amino acid pathway need to be increased. Also suggested by the FBA (Fig. S1), the flux through the 3-dehydroquinate dehydratase had to be increased 60-fold to maximize MA production. Unlike the self-standing enzymes from bacteria and plants that are in charge of converting 3-dehydroxy-D-arabino-heptulose-7-phosphate (DAHP) to 5-enolpyruvyl-3-shikimate phosphate (EPSP), *S. cerevisiae* ARO1 is a pentafunctional enzyme with the D subunit catalyzing the conversion of shikimate from DHS. By aligning the amino acid sequence of ARO1D with the self-standing DHS dehydrogenases from several organisms (Fig. S3), it was concluded that the residues K1370 and D1409 were the substrate binding site and the catalytic site, respectively. A panel of plasmids was subsequently created to enhance the synthetic flux towards DHS, but halt ARO1 from catalyzing DHS further into shikimate (Fig. S4). Among a number of mutants, ARO1<sub>D1409A</sub> (InvSc1 MA4) showed the best performance; its expression under the constitutive *gpd* promoter increased the production of MA to 235 mg L<sup>-1</sup> (strain InvSc1 MA4). To ensure that no carbon was being diverted to shikimate, we further deleted both copies of *aro1* (InvSc1 MA8 in Fig. 2A), but the MA production unexpectedly decreased to 25 mg L<sup>-1</sup>. The specific growth rate of

this *aro1* deletion mutant was 30% less than that of the wild type InvSc1, with 55% less biomass yield, suggesting that the decreased fitness of the deletion strain led to reduced MA production (Table S4). 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 InvSc1 $\Delta$ *pdcl* (resulting in InvSc1 MA9 and InvSc1 MA10, respectively). The MA titers decreased by around 60% and 74%, respectively (Fig. 2A). Recirculation of pyruvate to PEP has been successfully applied in *Escherichia coli* to increase the yield of aromatic compounds (18, 19), whereas in *S. cerevisiae*, the failure of this strategy might be due to low availability of pyruvate in the cytosol due to compartmentalization (20).

Despite the aforementioned genetic manipulations, the production of MA was still limited by the accumulation of the intermediate protocatechuic acid (PCA). The enzyme PCA decarboxylase is known to be oxygen sensitive (12, 21), whereas catechol dioxygenase (CDO) needs oxygen as a substrate. We observed that the conversion of PCA to catechol increased more than 2-fold when growing the cells in an oxygen-limited culture (Fig. 2B), suggesting that a rigorous control of the oxygen during fermentation had to be implemented. The strain InvSc1 MA4 was selected for fermentation in a 250 mL reactor with the dissolved oxygen (DO) spanning from 2% to 40% during the first 24 hours of growth (Fig. 2C). The highest MA production occurred in a mid-oxygen environment (10% < DO < 20%). This improved the ratio of MA to PCA to 2.5, which is almost 5-fold higher than the previously highest MA yeast producer (13). The highest MA titer reached 559.5 mg L<sup>-1</sup>, representing a 4-fold improvement in both titer and yield over strain MuA12. This improvement is attributed to different host ploidy, overexpression of the novel mutant ARO1<sub>D1409A</sub>, and by reducing the PCA bottleneck with a controlled oxygen environment in the fermentation. The yield of 14 mg<sub>MA</sub> g<sub>glucose</sub><sup>-1</sup> represents the highest one among the aromatic amino acid-based metabolites that have been produced in yeast in batch fermentation.

The fermentation broth was subsequently hydrogenated in a three-electrode electrochemical cell (Fig. 3A). Electrocatalysis was preferred over conventional high-pressure hydrogenation as hydrogen is produced *in situ* by water splitting, the reaction occurs under ambient temperature and pressure, and the charge on the electrode surface can mitigate poisoning (22). In this configuration, hydrogen production and MA hydrogenation take place simultaneously at the cathode (Eq. 1-2), allowing a seamless ECH.



Lead (Pb) was chosen as a catalyst based on its Earth abundance, low cost, and potential stability in the presence of sulfur (23). 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 coming from cellular metabolism and lysis. The electrocatalytic hydrogenation was then allowed to proceed at room temperature and atmospheric pressure for 1 hour by applying a potential of -1.5 V vs. Ag/AgCl on a 10 cm<sup>2</sup> lead rod, resulting in 95% MA conversion with 81% selectivity to HDA. To assess the stability of the catalyst in the fermentation media (in the presence of all potential poisons), five successive one-hour electrocatalytic batch reactions were performed (Fig. 3B). 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/sulfuric acid electrolyte was used to accurately control ionic strength and to maintain a constant ionic conductivity. Acidic conditions favored the selective formation of HDA, especially for reaction times below 30 min (Fig. S5). Further <sup>1</sup>H nuclear magnetic resonance (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 (Fig. S6). These undesired reactions were enhanced when increasing pH and/or the applied cathodic voltage (Fig. S7). A potential of -1.5 V and a pH of 2.0 offered a compromise between conversion and selectivity.

Conditions optimized with the model solutions were found to also enhance the hydrogenation of the fermentation broth (Fig. 3C). 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%). It appears that the broth's impurities prevent the formation of decomposition products. Ultimately, this integrated biological and electrochemical process presents several synergies: the residual salts and acidity of the fermentation broth favors the electrocatalytic reaction and the generated biogenic impurities enhance the selectivity to the desired HDA product.

In the pursuit of a fully integrated process, from glucose to a commercially viable product, the polymerization of HDA was studied. HDA has been previously used as a precursor to generate dodecanedioic acid (24), a monomer of nylon 6,12, and to produce polyester ethers with biomedical applications (25). Here we synthesized a bio-based UPA 6,6 through a polycondensation reaction utilizing HDA and HMDA. This polyamide has the advantage of having an extra double bond in its backbone that can be used to incorporate additional functionality. The corresponding saturated nylon 6,6 was synthesized using adipic acid and HMDA through the same procedure in an attempt to compare the conventional petrochemical-based nylon 6,6 and the bio-based UPA 6,6 polymers. The obtained UPA 6,6 consisted of a transparent, partly crystallized material that possessed physical and chemical properties that are comparable to petrochemical nylon (Fig. 4, Table S5, Fig. S8-S14). Utilizing the same synthesis technique, polymers based on blends of HDA and adipic acid were also achieved (Fig. S14) to potentially enable different levels of functionality.

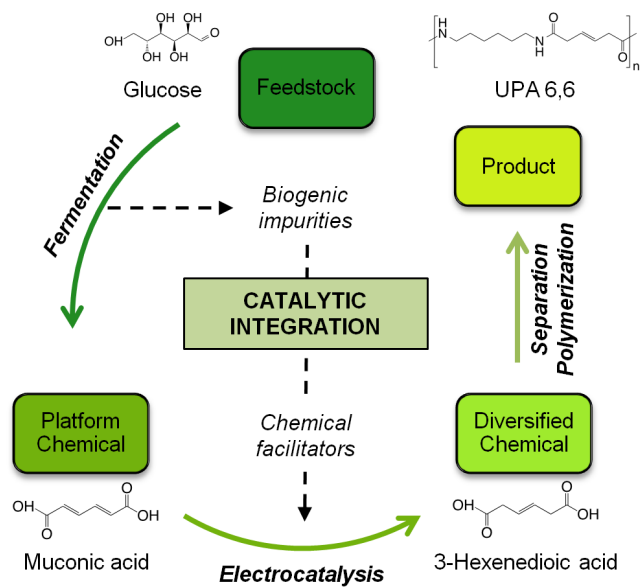
This work presents a strategy to bridge biological and chemical catalysis and streamline their integration in processes with optimized energy and resource utilization. We anticipate that this strategy will facilitate the incorporation of fermentation and catalytic hydrogenation for a broad range of reactions.

## References and Notes:

1. N. Hernandez, R. C. Williams, E. W. Cochran, *Org. Biomol. Chem.* **12**, 2834-2849 (2014).
2. S. Choi, C. W. Song, J. H. Shin, S. Y. Lee, *Metab. Eng.* **28**, 223-239 (2015).
3. A. Corma, S. Iborra, A. Velty, *Chem. Rev.* **107**, 2411-2502 (2007).
4. J. G. Linger, D. R. Vardon, M. T. Guarnieri, E. M. Karp, G. B. Hunsinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12013-12018 (2014).
5. D. R. Vardon, M. A. Franden, C. W. Johnson, E. M. Karp, M. T. Guarnieri *et al.*, *Energy Environ. Sci.* **8**, 617-628 (2015).

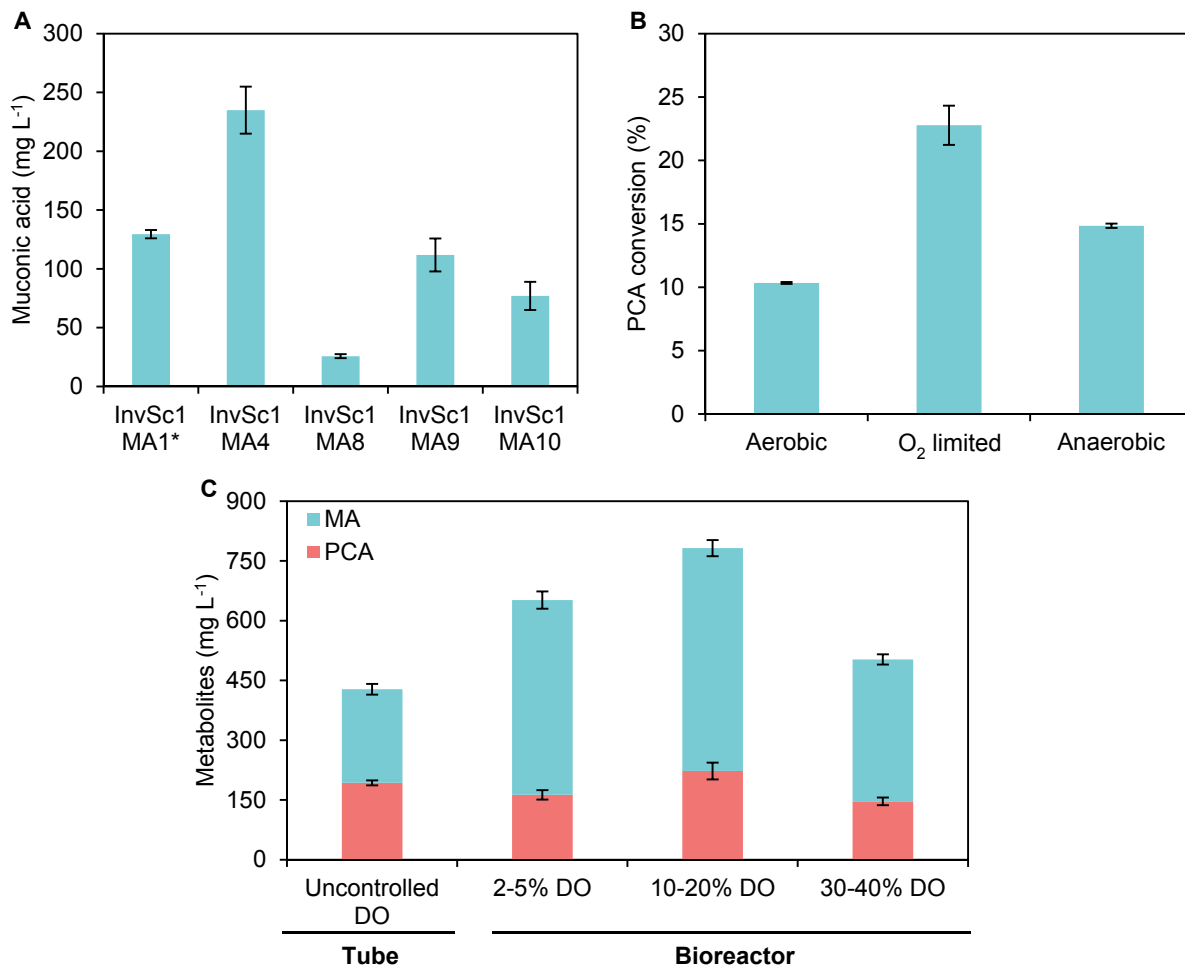
6. T. J. Schwartz, B. J. O'Neill, B. H. Shanks, J. A. Dumesic, *ACS Catal.* **4**, 2060-2069 (2014).
7. B. J. Nikolau, M. A. D. N. Perera, L. Brachova, B. Shanks, *Plant J.* **54**, 536-545 (2008).
8. Z. Zhang, J. E. Jackson, D. J. Miller, *Bioresource Technol.* **99**, 5873-5880 (2008).
9. T. J. Schwartz, R. L. Johnson, J. Cardenas, A. Okerlund, N. A. Da Silva *et al.*, *Angew. Chem. Int. Ed.* **53**, 12718-12722 (2014).
10. M.-J. In, D. C. Kim, H. J. Chae, *Biotechnol. Bioprocess Eng.* **10**, 85-90 (2005).
11. T. W. Jeffries, *Curr. Opin. Biotechnol.* **17**, 320-326 (2006).
12. C. Weber, C. Bruckner, S. Weinreb, C. Lehr, C. Essl *et al.*, *Appl. Environ. Microbiol.* **78**, 8421-8430 (2012).
13. K. A. Curran, J. M. Leavitt, A. S. Karim, H. S. Alper, *Metab. Eng.* **15**, 55-66 (2013).
14. M. A. H. Luttki, Z. Vuralhan, E. Suir, G. H. Braus, J. T. Pronk *et al.*, *Metab. Eng.* **10**, 141-153 (2008).
15. K. Li, M. R. Mikola, K. M. Draths, R. M. Worden, J. W. Frost, *Biotechnol. Bioeng.* **64**, 61-73 (1999).
16. Y.-Y. Cui, C. Ling, Y.-Y. Zhang, J. Huang, J.-Z. Liu, *Microb. Cell Fact.* **13**, 21 (2014).
17. J. Du, Y. Yuan, T. Si, J. Lian, H. Zhao, *Nucleic Acids Res.* **40**, e142 (2012).
18. H. Zhang, B. Pereira, Z. Li, G. Stephanopoulos, *Proc. Natl. Acad. Sci. U.S.A.*, **112**, 8266-8271 (2015).
19. R. Patnaik, J. C. Liao, *Appl. Environ. Microbiol.* **60**, 3903-3908 (1994).
20. J. L. Avalos, G. R. Fink, G. Stephanopoulos, *Nat. Biotechnol.* **31**, 335-341 (2013).
21. Z. He, J. Wiegel, *J. Bacteriol.* **178**, 3539-3543 (1996).
22. J. M. Chapuzet, A. Lasia, J. Lessard, *Electrocatalytic hydrogenation of organic compounds*. In: *Electrocatalysis*, J. Lipkowski, P. N. Ross, Eds. (Wiley-VCH, New-York, 1998), pp. 155-196.
23. R. W. Joyner, K. Kishi, M. W. Roberts, *Proc. R. Soc. London, Ser. A* **358**, 223-241 (1978).
24. J. W. Frost, J. Millis, Z. Tang. Patent WO2010085712, 2010.
25. D. A. Olson, S. E. A. Gratton, J. M. DeSimone, V. V. Sheares, *J. Am. Chem. Soc.* **128**, 13625-13633 (2006).

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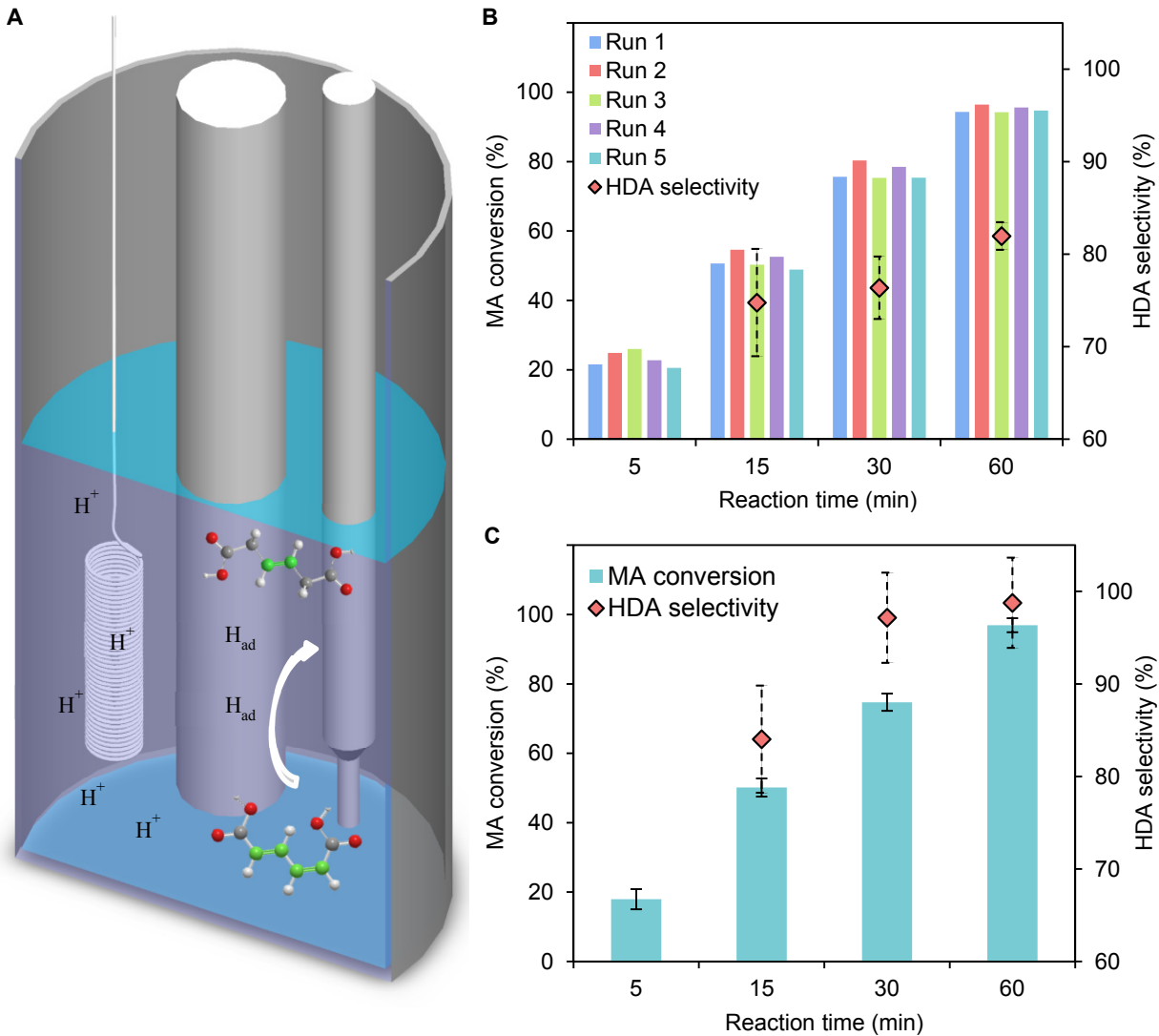
**Fig. 1. Integrated conversion of glucose to UPA 6,6.** The catalytic integration was enabled by the compatibility of the process parameters. Replacing conventional high-pressure hydrogenation by direct ECH promoted a seamless flow between the processes. This removed the need of intermediate separation and allowed the use of the broth water, salts, and impurities as an electrolyte and hydrogen source.





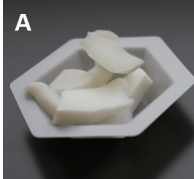
**Fig. 2. Characterization of MA production in engineered *InvSc1* strains.** (A) MA accumulation in strains grown in glass tubes containing 15 mL of selective media. Maximum MA acid was observed at 96 h of aerobic fermentation. (B) PCA decarboxylase activity assay under different oxygen environments. The strain *InvSc1* pRS414 *aroY* was cultured in three different oxygen conditions and spiked at 24 h with 1 mM PCA. The conversion percentages were calculated based on the samples collected at 18 h after PCA supplementation. (C) *InvSc1*MA4 in mini reactor fermentations with controlled dissolved oxygen. The highest MA accumulation was observed when the dissolved oxygen was maintained between 10 to 20% during the first 24 h. After this period, the dissolved oxygen was set to 20% until the end of the fermentation (4 days).

\* SC-LH media supplemented with 2 % glucose; 4% glucose was used in all the other cases.

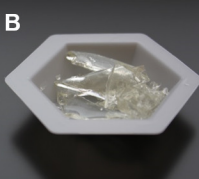


**Fig. 3. Electrocatalytic hydrogenation of MA to HDA directly in the fermentation broth.** The hydrogen necessary for the reaction is generated *in situ* ( $H_{ad}$ ) at the surface of the Pb electrode. **(A)** Electrocatalytic single-cell reactor for conversion of MA to HDA. Atoms in the molecules are color-coded: grey: carbon; white: hydrogen; red: oxygen; blue: nitrogen; green: carbon-carbon double bond. The reaction was performed under 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 repeating the reaction five successive times (runs 1-5). **(C)** MA and HDA conversion and selectivity for the ECH of the doped (pH 2.0, -1.5 V) fermentation broth.

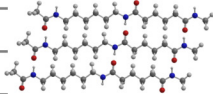
	Nylon 6,6	UPA 6,6
$M_n$ [Da]	17,800	12,200
PDI	2.0	3.36
Melting Temp. [°C]	250	105-190
$Q^*$ [Å <sup>-1</sup> ]	4.4	4.7
$G'$ [MPa]	-	18.9
$G''$ [MPa]	-	6.24
$G_c^*$ [°C]	-	60

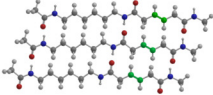


**A**



**B**





**Fig. 4. Comparison of the synthesized polymers.** The table summarizes nylon 6,6 and the UPA 6,6 properties, where  $M_n$  indicates the number average molecular weight, PDI: polydispersity,  $Q^*$ : primary diffraction peak,  $G'$ : storage modulus,  $G''$ : loss modulus, and  $G_c^*$ : crossover modulus. **(A)** Nylon 6,6 and **(B)** UPA 6,6, for which petroleum-based adipic acid was substituted with HDA ; atoms in both molecular structures are color-coded for easier identification. Grey: carbon; white: hydrogen; red: oxygen; blue: nitrogen; green: carbon-carbon double bond.