Biosynthesis of Furfurylamines in Batch and Continuous Flow by Immobilized Amine Transaminases

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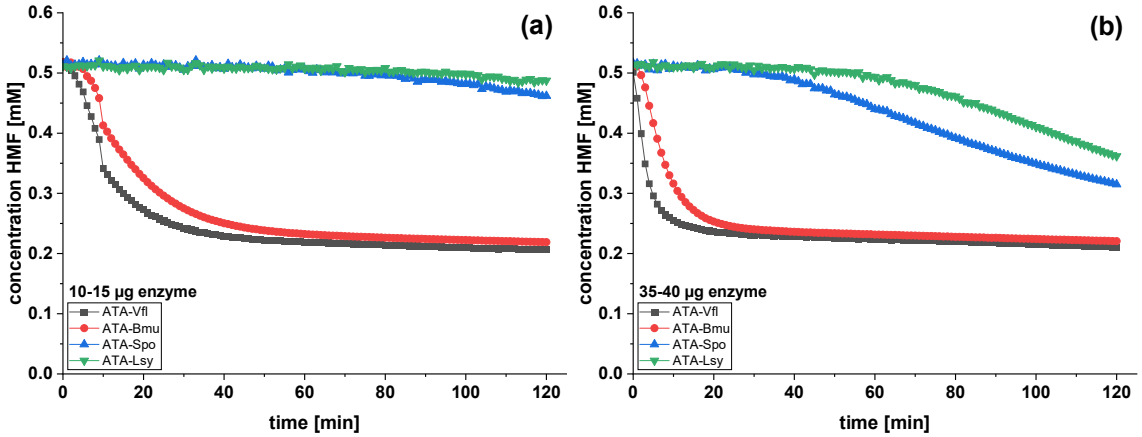

Figure S1. Amination of HMF by soluble ATAs. Soluble ATAs (ATA-Vfl (■), ATA-Bmu (●), ATA-Spo (▲), and ATA-Lsy (▼)) were used in two different amounts (10–15 µg **(a)** or 35–40 µg **(b)**) for the amination of HMF to HMFA in a 150 µL reaction solution (50 mM Tris pH 8.0, 1 mM HMF, 25 mM alanine (L- or D-alanine for (*S*)- or (*R*)-selective ATAs, respectively), 0.1 mM PLP) in UV microtiter plates at 37 °C, which was followed at 283 nm in the TECAN reader. A maximum of 0.5 mM HMF could be detected as the absorbance was above the maximum absorbance of the TECAN reader (>3.5 AU).

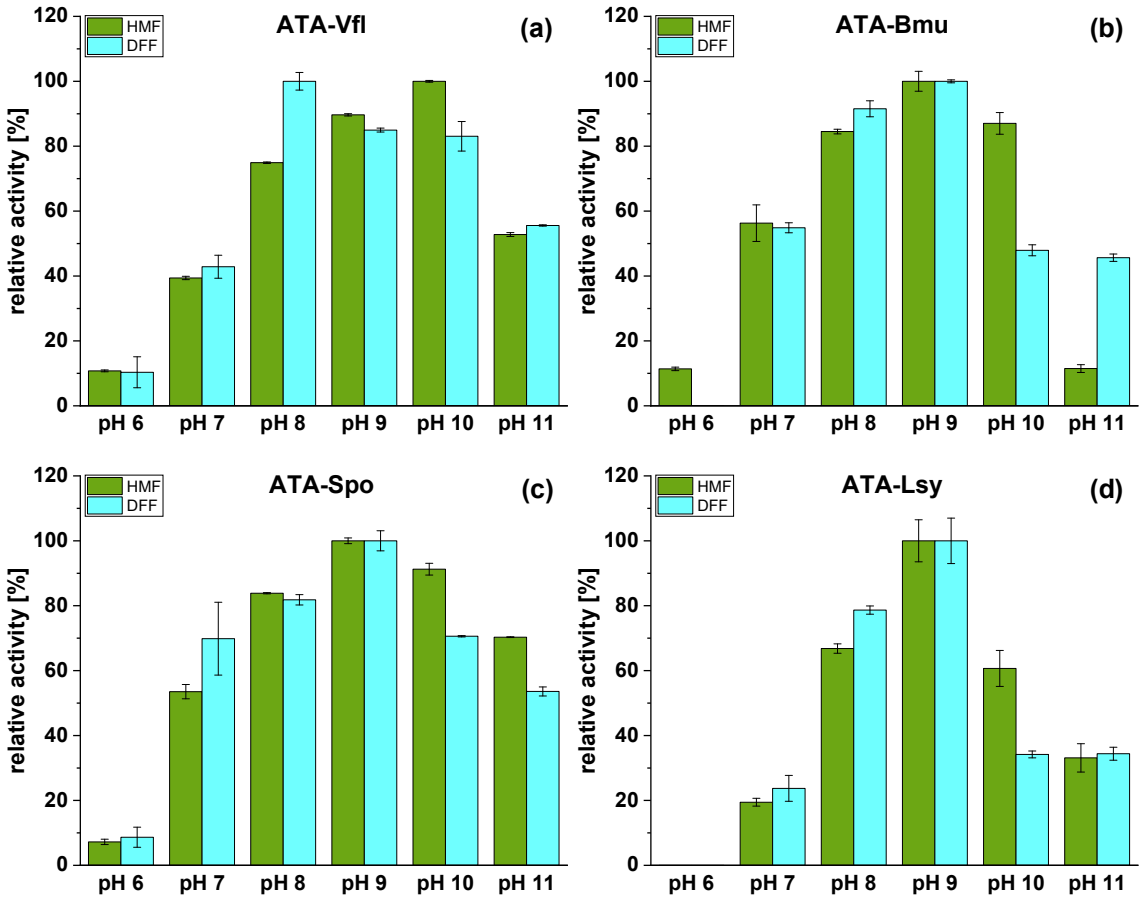

Figure S2. Relative activity of different ATAs at different pH with HMF and DFF as substrate. The specific activity of soluble ATAs (ATA-Vfl **(a)**, ATA-Bmu **(b)**, ATA-Spo **(c)**, ATA-Lsy **(d)**) was determined at different pH (6 (50 mM phosphate buffer), 7-9 (50 mM Tris buffer), 10-11 (50 mM CAPS buffer)) at 37 °C with HMF (green) and DFF (blue) as substrate and alanine as co-substrate in 150 µL reaction solution (50 mM buffer, 0.5 mM HMF, 5 mM alanine (L- or D-alanine for (*S*)- or (*R*)-selective ATAs, respectively), 0.1 mM PLP, 1 % DMSO) each containing 8-16 µg enzyme each.

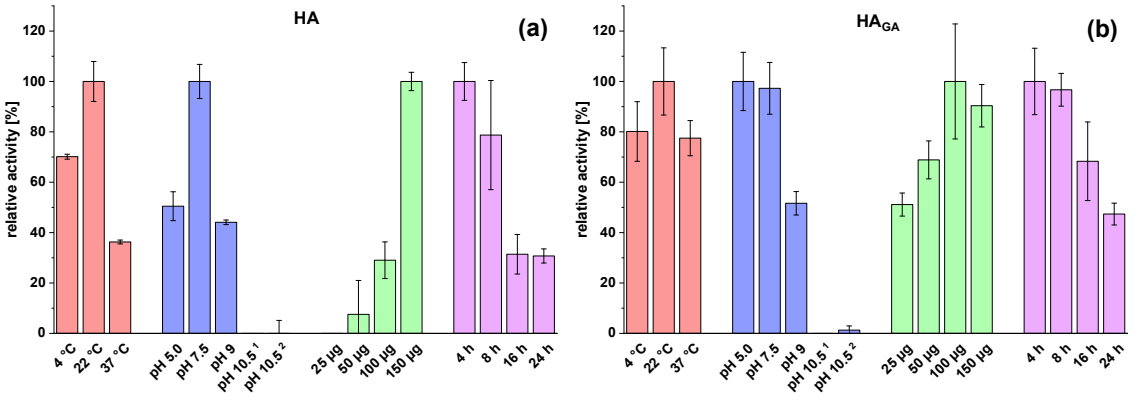

Figure S3. Analysis of various immobilization conditions for ATA-Spo. ATA-Spo was immobilized on HA-beads **(a)** and HA$_{GA}$-beads **(b)** at different temperatures (red), at different pH (blue), with different enzyme amounts per mg bead (green), and for different immobilization durations (pink). An appropriate volume of enzyme solution (depending on the amount of enzyme desired) was added to the beads (5 mg each) and immobilization was allowed under the stated conditions, while the standard conditions (50 mM bicine buffer, pH 9.0, 20 µM ATA-Spo solution, 100 µg enzyme per mg bead, 37 °C, 24 h) were used in all series of experiments, except for the varied conditions indicated. Subsequently, the beads were washed and blocked, and the specific activity of the immobilized enzyme was determined as described in the methods. In each series of experiments (temperature, pH, enzyme amount, duration, and additives), the condition that yielded the highest specific activity of the immobilizates was set at 100%, and the others in that series were related to it. All immobilizations were performed in triplicate, and the error bars represent the standard deviation. pH 10.5[1]: glycine-sodium hydroxide buffer, pH 10.5; pH 10.5[2]: CAPS buffer, pH 10.5.

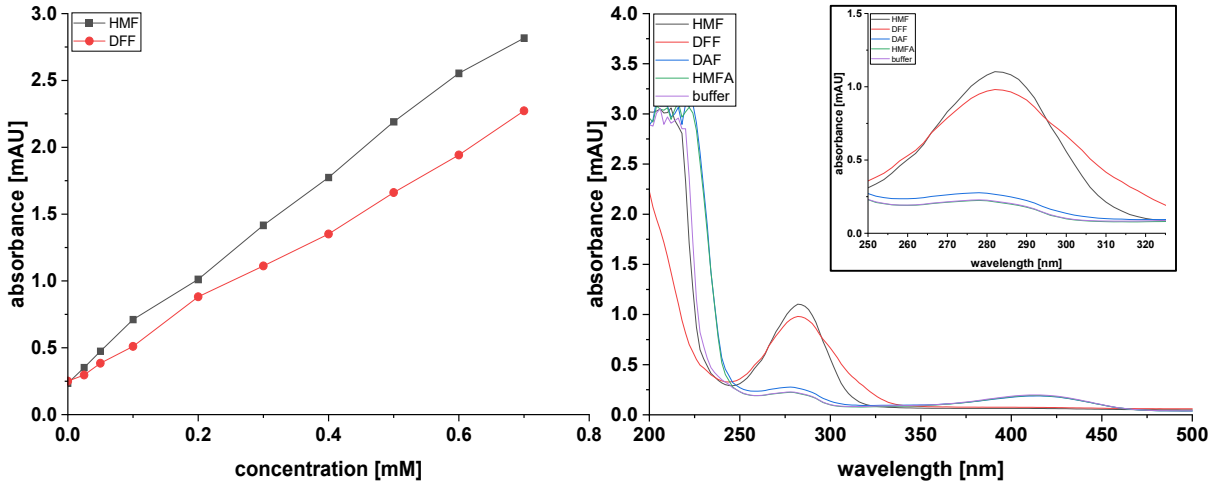

Figure S4. Characterization of HMF, HMFA, DFF, and DAF. Both HMF (■) and DFF (●) were dissolved in Tris buffer (50 mM, 0.5% DMSO, 0.1 mM PLP, pH 8.0) and analyzed (100 µL each) in UV-microtiter plates (UV-Star®, Greiner Bio-One) in the TECAN reader. **(a)** HMF and DFF were measured at different concentrations (0–0.7 mM) at 283 nm. The linear fit function was y=3.726x + 0.2824 (R^2=0.9981) and y=2.8609x + 0.2438 (R^2=0.9984), and the calculated extinction coefficient was 14.922 $mM^{-1}cm^{-1}$ and 10.610 $mM^{-1}cm^{-1}$ for HMF and DFF, respectively. **(b)** The absorbance spectra of HMF, HMFA, DFF, and DAF (1 mM each) were analyzed from 200–500 nm in 2 nm steps. The box represents the enlargement from 250–325 nm and shows the absorbance maximum at 282-284 nm of HMF and DFF, leading to detection of both at 283 nm.

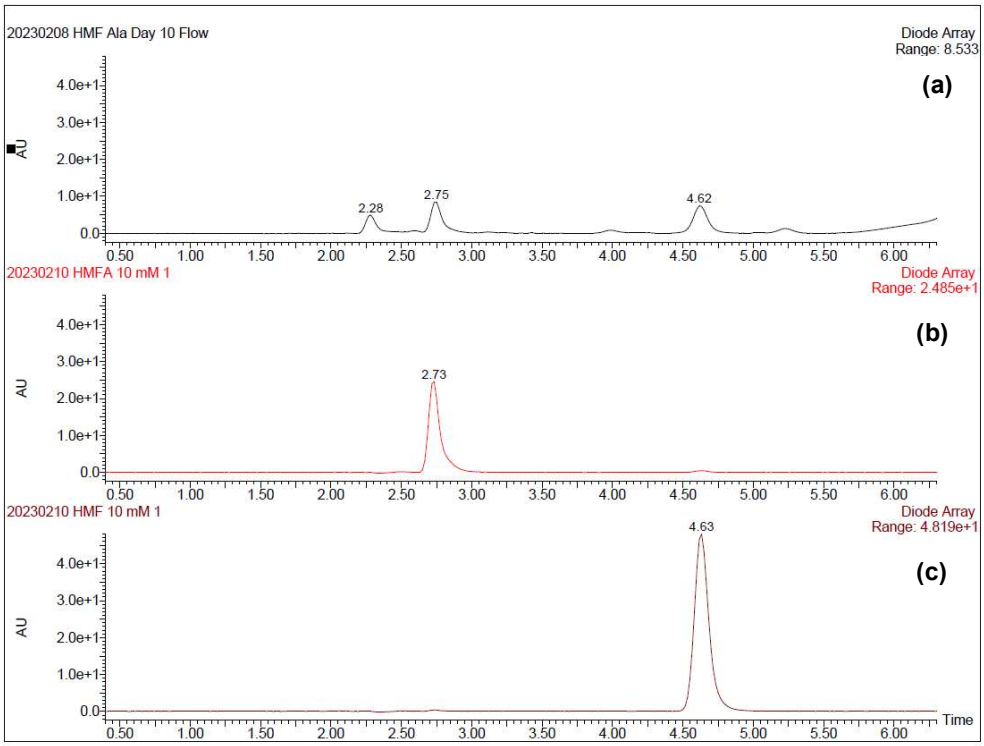

Figure S5. HPLC Analysis of HMFA and HMF. (**a**) Chromatogram of the continuous flow reaction of 10 mM HMF with 500 mM L-alanine with ATA-Spo after 10 days of continuous use. Exemplary chromatogram for the detection of HMFA (RT 2.75 min) and HMF (RT 4.62 min) with a visible L-alanine peak (RT 2.28 min). (**b**) Chromatogram of commercial HMFA (10 mM), RT 2.73 min. (**c**) Chromatogram of HMF (10 mM), RT 4.63. All chromatograms used the method described in the Materials and Methods chapter.