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Biotransamination of Furan-Based Aldehydes with Isopropylamine: Enzyme Screening and pH Influence

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Furan-based amines are highly valuable compounds which can be directly obtained via reductive amination from easily accessible furfural, 5-(hydroxymethyl)furfural (HMF) and 2,5-diformylfuran (DFF). Herein the biocatalytic amination of these carbonyl derivatives is disclosed using amine transaminases (ATAs) and isopropylamine (IPA) as amine donors. Among the different biocatalysts tested, the ones from *Chromobacterium violaceum* (Cv-TA), *Arthrobacter citreus* (ArS-TA), and variants from *Arthrobacter* sp. (ArRmut11-TA) and *Vibrio fluvialis* (Vf-mut-TA), afforded high levels of product formation (>80%) at 100–200 mM aldehyde concentration. The transformations were studied in terms of enzyme and IPA loading. The pH influence was found as a key factor and attributed to the imine/aldehyde equilibrium that can arise from the high reactivity of the carbonyl substrates with a nucleophilic amine such as IPA.

Introduction

In recent decades, lignocellulosic biomass has stood out as a potential carbon source, which can replace petrochemical products due to its diversity and availability. Among the many different types of compounds that can be obtained from raw biomass, amino derivatives present a paramount relevance, given their wide industrial applications.[1] In this context, furan-based amines (Figure 1a) such as furfuryl amine (FAm, **1a**),[2] 5-hydroxymethyl-2-furfuryl amine (HMFA, **2a**),[3] and 2,5-bis(aminomethyl)furan (BAMF, **3a**)[3] are excellent examples of valuable bio-based compounds. Hence, FAm is utilized as precursor of furmethide,[4] used to treat glaucoma, or pyridostigmine drugs[5] employed against myasthenia gravis. HMFA is a useful intermediate in the synthesis of bioactive molecules,[6] and BAMF is an interesting monomer for various functionalized polymers.[7]

Among the plethora of synthetic methodologies to access these amine derivatives, the most commonly used is the metal-catalyzed reductive amination[2b,3] of the easily accessible carbonyl precursors furfural or 5-(hydroxymethyl)furfural (HMF),[8] combining ammonia with hydrogen or a reducing agent. For instance, recent contributions have reported the use of different metals, being cobalt,[9] nickel,[10] and ruthenium[11] the most employed ones. However, these methods present serious drawbacks such as the use of elevated hydrogen pressures, high temperatures, and harmful organic solvents, normally affording a mixture of products due to the reactivity of the imine intermediate formed from the carbonyl moieties and ammonia.

With these considerations in mind, milder catalytic alternatives such as biocatalysis[12] are highly appealing since they can provide sustainable solutions to solve synthetic problems, in particular the selective synthesis of amines from reactive carbonyl compounds.[13] Among the biocatalysts that have emerged with tremendous potential in the field, amine trans-

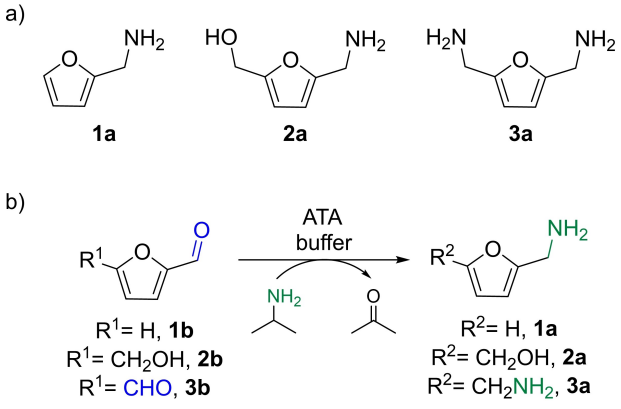

Figure 1. a) Structure of furfuryl amine (**1a**), 5-hydroxymethyl-2-furfuryl amine (**2a**), and 2,5-bis(aminomethyl)furan (**3a**). b) General scheme of the biotransamination reactions over furan-based aldehydes studied in this contribution.

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aminases (ATAs) can be highlighted.[14] These enzymes catalyze the amination of ketones or aldehydes into the corresponding (chiral) amines at expenses of a cheap amine donor (e.g., isopropylamine, IPA),[15] used in substantial molar excess to drive the equilibrium into the desired synthetic direction.[16] Furthermore, low boiling points of IPA and the obtained co-product (acetone), facilitate the reaction work-up in synthetically useful procedures.[16b] Specifically, ATAs from *Chromobacterium violaceum* (Cv-TA)[17] and *Aspergillus terreus* (At-TA)[18] have been described as very efficient catalysts to synthesize FAm[19] and HMFA[19a,20] in plain buffer, or alternatively using bio-based organic solvents[21] and designed deep eutectic solvents (DES)[20,22] to improve substrate solubility. Very recently, thermostable mutants from At-TA have shown to be very effective in processes at high furfural[23] and HMF[23b,24] concentrations. However, most of these transformations were performed using an excess of alanine as amine donor, and in the case of At-TA-catalyzed transformations, the expensive D-alanine had to be employed.

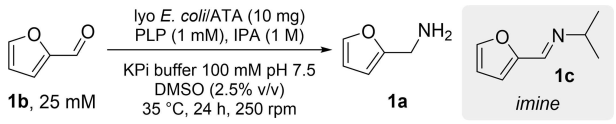
Following our interest in the biocatalytic modification of bio-based furan derivatives,[25] herein we present the results obtained in the biotransamination of furan aldehydes **1b**–**3b** using IPA as an easily accessible amine donor in aqueous media (Figure 1b). Despite the system limitations due to the high reactivity of these derivatives, this study also provides simple solutions to improve the productivity of these reactions.

Results and Discussion

Biotransamination of furfural (1b)

Furfural (**1b**, 25 mM) has been used as the first substrate to study the transamination with a set of ATAs. Thus, 28 commercial enzymes from Codexis and 9 recombinant *E. coli* cells encoding an ATA were assessed. For the latter, cells were cultivated after induction procedure and used as dry cell powder after lyophilization. The reaction conditions were defined based on previous reports,[26] using potassium phosphate (KPi) buffer 100 mM pH 7.5 and dimethylsulfoxide (DMSO, 2.5% v/v) for **1b** solubilization reasons, and IPA (40 equiv.) as amine donor. The enzymatic reactions were carried out in parallel with one blank reaction (sample without ATA). Interestingly, in the absence of ATA, the expected peak corresponding to aldehyde **1b** was not detected, while a new signal was observed in the GC chromatogram (see Figures S2 and S3 in SI). This can be explained by the high reactivity of IPA (in great excess to shift the equilibrium towards the amine synthesis), with the aldehyde group of furfural, thus leading to the formation of the imine compound **1c** (Scheme from Table 1, as demonstrated by NMR studies, see SI). The presence of this singular peak has been interpreted as residual substrate in all enzymatic reaction samples, and its area compared to the one corresponding to furfuryl amine, providing the conversion values for each tested ATA, since furfural signal was not detected after the basic work-up of the reaction crudes.

Table 1. Selected furfural biotransamination results using overexpressed ATAs in *E. coli* after 24 h at 35 °C.



Entry	ATA	**1a** (%)[a]	**1b** (%)[a]	**1c** (%)[a]
1	Cv-TA	>99	<1	<1
2	ArS-TA	>99	<1	<1
3	ArRmut11-TA	>99	<1	<1
4	Bm-TA	>99	<1	<1
5	BmS119G-TA	>99	<1	<1
6	Vf-mut-TA	>99	<1	<1
7	ArR-TA	57	<1	43
8	Vf-TA	66	<1	34
9	At-TA	4	<1	96

[a] Compound percentage values were calculated by GC analyses of the crude reaction mixtures after basic extraction using calibration curves (see Section VI.1 in SI).

Among the 28 commercial amine transaminases, only 3 afforded a conversion into **1a** lower than 10%, while the rest catalyzed quantitative formation of the desired product (Table S3 in SI).[27] Besides that, several made-in-house overexpressed ATAs also allowed to obtain quantitative conversions into furfuryl amine (Table 1). Thus, the best results were attained with ATAs from *Chromobacterium violaceum* (Cv-TA),[17] *Arthrobacter citreus* (ArS-TA),[28] a variant from *Arthrobacter* sp. (ArRmut11-TA),[29] *Bacillus megaterium* (Bm-TA),[30] its variant S119G (BmS119G-TA),[31] and a variant from *Vibrio fluvialis* (Vf-mut-TA).[32] Other enzymes such as the ones from *Arthrobacter* sp. (ArR-TA),[33] *Vibrio fluvialis* (Vf-TA),[34] and *Aspergillus terreus* (At-TA),[18] afforded lower product formation (4-66%).

In a new set of experiments (Figure 2), biotransamination of **1b** was performed at higher substrate concentrations (50–200 mM). The reactions were done with the same amount of the lyophilized *E. coli* cells overexpressing the best ATA candidates (entries 1–6, Table 1), while keeping the remaining reaction conditions constant. Among the panel of enzymes,

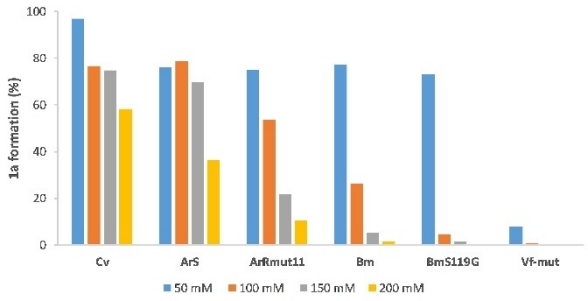


Figure 2. Transamination of furfural (**1b**) with increasing concentrations of the substrate (50–200 mM). Amine **1a** percentage values were determined by GC analyses of the crude reaction mixtures after basic extraction using calibration curves (see Section VI.1 in SI).

only Cv-TA and ArS-TA catalyzed efficiently the transformation into **1a** with a percentage of 70–75% at 150 mM of **1b**. Significant drops of product formation were attained for transamination reactions catalyzed by ATAs from *Bacillus megaterium* and *Vibrio fluvialis*.

Due to the high reactivity of aldehyde compounds with proteins, a possible ATA inactivation at high **1b** concentrations cannot be ruled out. To overcome this, the stepwise addition of the inhibiting substrate can be implemented. Thus, as a proof of concept, an experiment with *E. coli*/ArS-TA was designed, adding **1b** sequentially (50 mM each addition), after 0, 3, 6, and 9 h (final concentration: 200 mM). After 24 h, the conversion into amine **1a** was 95%, largely improving the previous result (36%, Figure 2) at 200 mM. This experiment opens the door for future process optimization.

Biotransamination of HMF (2b)

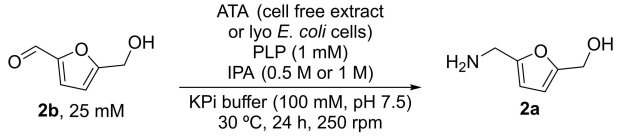
Subsequently, the transamination of hydroxy aldehyde **2b** (25 mM) was studied under similar conditions as previously described (KPi buffer 100 mM, pH 7.5),[35] using crude cell free extracts (CFE) or *E. coli* lyophilized cells with the best candidates found for furfural. The concentration of IPA was set at 0.5 M for enzymes used as cell free lysate due to possible inhibition, while 1 M was selected for enzymes used as lyophilized powder. After incubation at 30 °C and 250 rpm for 24 h, the reactions were stopped by addition of trifluoroacetic acid (TFA) in water (10% v/v) and the samples were analyzed by reverse phase HPLC.[19a] Due to the employed acidic treatment, the imine between IPA and HMF was not detected in the chromatograms, opposite to the furfural samples, studied by GC after basic work-up.

Several enzymes rendered good conversions under the tested conditions, obtaining in many cases quantitative yields (Table 2). Thus, Cv-TA, ArS-TA, ArRmut11-TA, Bm-TA, Vf-mut-TA, and Vf-TA were selected as the best candidates for studying the transformations at higher substrate concentrations.

Interestingly, when the reactions were tested with 50 mM of **2b** while keeping the same enzyme loading and employing 0.5 M of IPA (Figure S1 in SI), it was observed that full conversions for all 6 candidates were still attained. Given the promising results obtained with HMF as substrate, we decided to optimize the enzyme loading, i.e., the minimum ATA amount per mmol of substrate to obtain full conversion (Table S4 in SI). Thus, the conditions used for this study were 50 mM of **2b** and 0.5 M of IPA.

With the lowest enzyme/substrate loadings found for these ATAs and keeping the IPA excess constant (10 equiv.), HMF concentration was subsequently increased to 100 and 200 mM (Figure 3a). Under these conditions, the conversion considerably decreased at 200 mM of HMF for Cv-TA, Vf-TA, and Bm-TA (< 50%), while ArRmut11-TA, ArS-TA, and Vf-mut-TA provided values higher than 80%, with the last two ATAs giving nearly quantitative conversions. Additionally, the influence of the enzyme loading on the reaction conversion was investigated, by doubling the enzyme amount while keeping IPA concentration constant (500 mM, Figure 3b). Although complete conversions were obtained after increasing **2b** concentration from 50 to 100 mM, transformations rendered only 50–70% of amine **2a** at 200 mM of substrate. Such results probably are related to the reaction equilibrium, evidencing the need for a higher IPA excess since only 2.5 equiv. were employed, which might not

Table 2. HMF biotransamination results using ATAs after 24 h at 30 °C.



Entry	ATA	**2a** (%)[a]	**2b** (%)[a]
1	Cv-TA[b]	>99	<1
2	ArS-TA[b]	>99	<1
3	ArRmut11-TA[b]	>99	<1
4	Bm-TA[c]	>99	<1
5	Vf-mut-TA[c]	>99	<1
6	ArR-TA[b]	15	85
7	Vf-TA[c]	>99	<1
8	At-TA[c]	61	39

[a] Conversion values were calculated by reverse phase HPLC analyses of the crude reaction mixtures using calibration curves (see Section VI.2 in SI). [b] Used as CFE (240 μL). [c] Used as lyophilized whole cells (5 mg).

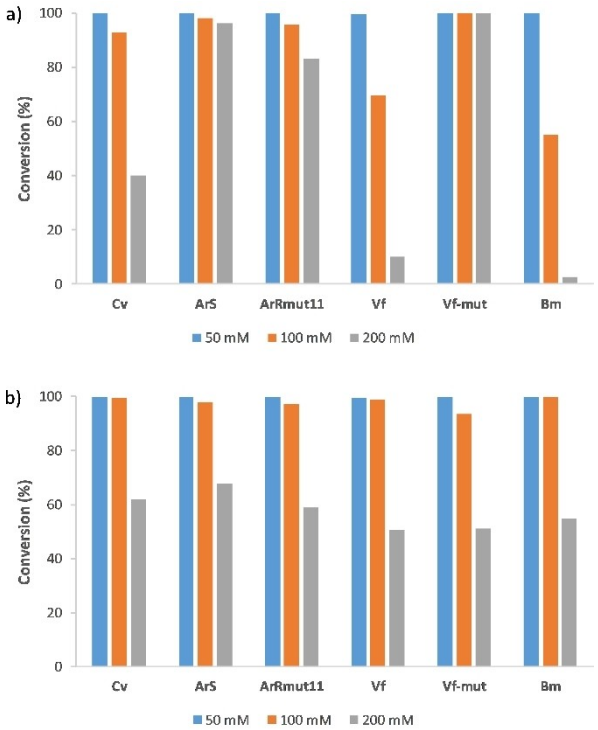


Figure 3. Results in the HMF (**2b**) biotransamination (50, 100, and 200 mM) using: a) the enzyme quantity in the ratio specified in Table S4 in SI and IPA (10 equiv.) as amine donor; and b) doubling the amount of ATA while keeping constant IPA concentration (500 mM), for 24 h at 30 °C and 250 rpm. Conversions were determined using reverse phase HPLC analyses of the crude reaction mixtures using calibration curves (see Section VI.2 in SI).

be enough to completely shift the reaction towards the product formation.

Biotransamination of DFF (3b)

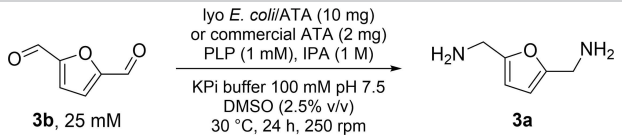
Before performing the enzymatic screening, solubilization tests were made with amine **3a**, observing that it could not be extracted with different organic solvents due to its high solubility in water. Thus, as in the case of amine **2a**, reverse phase HPLC was selected as analytical method for the reaction monitoring to measure product concentrations in **3b** biotransaminations.

It should be highlighted that there is limited information that can be found in the literature where DFF has been studied with ATAs.[19a] Therefore, the complete panel of transaminases including the commercial ones was screened, for selecting the best performing enzymes (Table 3 and Table S5 in SI). We used previously established reaction conditions, selecting IPA (1 M) as amine donor in KPi buffer 100 mM pH 7.5 and adding DMSO (2.5% v/v) as co-solvent due to the relatively low solubility of DFF in water.

The blank reaction (without enzyme addition) confirmed again the total conversion of DFF in the reaction medium into the diimine derivative **3c** (absorbing at 280 nm, with a similar retention time as DFF), due to the high reactivity of IPA with the aldehyde groups of DFF in the aqueous medium (as demonstrated by NMR studies, see SI). Three different scenarios were observed in the progress of studied transformations (see Figure S8 in SI): a) in the cases that quantitative conversions into BAMF (detected at 210 nm) were attained, no residual diimine **3c** was detected; b) in reaction crudes with a very low formation of diamine **3a** (<10%), **3c** was observed at 280 nm as the main compound; and c) in the crudes with partial conversions into BAMF, few or none diimine residue was present and the mass balance of the reaction was not closed, indicating the presence of other possible intermediates. Moreover, the precipitation of a residue in the Eppendorf tubes was observed in these reaction crudes (see Figure S8C in SI), suggesting the formation of oligomeric or polymeric species.

Among the tested enzymes, 5 ATAs catalyzed the formation of diamine **3a** with >96% conversion (Table 3). Namely, commercial ATA-025 and ATA-251, and ArRmut11-TA, Bm-TA and BmS119G-TA. Hence, these enzymes were selected to study the effect of higher substrate concentrations (50 to 200 mM, Figure 4). Only Bm-TA afforded quantitative conversion into **3a** at 50 mM of DFF, meanwhile, the other ATAs provided the diamine in around 70% conversion. Unfortunately, higher concentrations provoked a significant drop in the formation of **3a**, indicating that these enzymes could not efficiently convert DFF at those loadings. DMSO amount was increased up to 5% v/v for 100 mM and 150 mM and 10% v/v at 200 mM of DFF, to ensure a better solubility in the reaction medium, but results did not improve. As previously commented, all the reaction crudes showed the disappearance of residual DFF (in the diimine form or as a solid residue). Another issue that should be considered is that the IPA amount was maintained in these experiments, and reaction equilibrium was then affected: starting from a 1:20 mol ratio of substrate:IPA (at 25 mM of substrate concentration, 2 reactive aldehyde groups per molecule), it was gradually decreased to 1:2.5 (at 200 mM of DFF).

Table 3. Selected DFF biotransamination results using ATAs after 24 h at 30 °C.



Entry	ATA	**3a** (%)[a]
1	Cv-TA	89
2	ArS-TA	91
3	ArRmut11-TA	>99
4	Bm-TA	>99
5	BmS199G-TA	>99
6	Vf-mut-TA	44
7	ArR-TA	22
8	Vf-TA	<1
9	At-TA	<1
10	ATA-251[b]	97
11	ATA-025[b]	>99

[a] Diamine **3a** percentage values were calculated by reverse phase HPLC analyses of the crude reaction mixtures using calibration curves (see Section VI.2 in SI). [b] Commercially available enzyme from Codexis Inc.

pH influence at higher substrate concentrations

It is clear that the transamination using IPA as amine donor with highly reactive substrates such as the ones studied in this contribution led to undesired reactions (Scheme 1). Thus, on one hand, the amine acceptor reacted with IPA to form the corresponding imine derivatives **1c**–**3c**, as detected in the previously described biotransformations (Scheme 1a). On the other hand, the desired amine product **1a** and **2a** formed during the reaction course could dimerize with the remaining substrate **1b** and **2b**, leading to the formation of additional

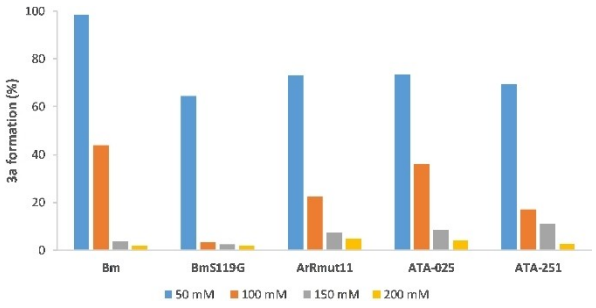
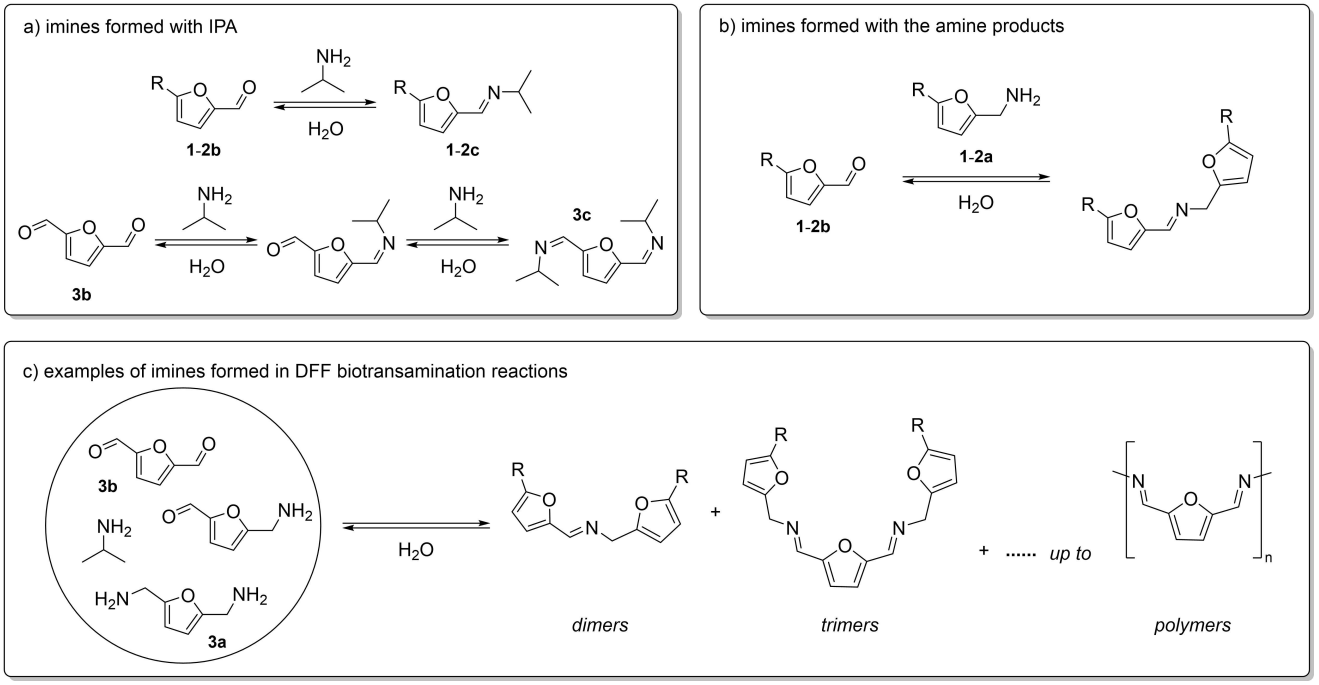


Figure 4. Transamination of DFF (**3b**) at increasing concentrations of the substrate (50–200 mM). Percentage values of **3a** were determined by reverse phase HPLC analyses of the crude reaction mixtures using calibration curves (see Section VI.2 in SI).



Scheme 1. Different reaction possibilities to form (poly)imines in the biotransamination of aldehydes **1 b**–**3 b** showed in the present study due to the: a) presence of IPA; b) presence of the amine products; and c) particular chemical structure of dialdehyde **3 b**.

imine species (Scheme 1b). Finally, in the special case of DFF, the overall picture was highly complex. Apart from the reaction equilibria with IPA (Scheme 1a), due to the existence of two reactive aldehyde groups per molecule, there could exist many other imine-type intermediates, even undergoing oligomerization and polymerization processes (Scheme 1c). In fact, this has already been noticed in previous contributions with transaminases[19a] and metal-catalyzed reductive aminations.[36]

Considering that the imine bond formation is reversible, especially in water, we investigated if pH could have an impact on these ATA-catalyzed processes. The reaction outcome could be hindered by the formation of the imines, thus decreasing the concentration of the aldehyde species available in the reaction medium. Since imines are easily formed at basic pHs, we envisaged that employing a more acidic reaction medium could improve these biotransaminations by avoiding, at least to some extent, the presence of undesired imine compounds. As a proof of concept, we repeated the ATA-catalyzed reactions with aldehydes **1 b** and **3 b** at different concentrations with the best enzymes previously found for each substrate (Figures 2 and 4), using KPi buffer 100 mM pH 6.5 as reaction medium (Figure 5).

In general, higher conversions towards the corresponding (di)amine products **1 a** and **3 a** were accomplished, thus supporting our assumption regarding the aldehyde/imine equilibrium and its influence on the target reaction. Especially remarkable were the positive results obtained with Cv-TA and ArS-TA (Figure 5a) and ATA-025 and ATA-251 (Figure 5b), in comparison with the results observed at pH 7.5 (Figures 2 and 4). However, for Bm-TA and its mutant, slightly lower conversions were achieved, likely related to the reduced stability or activity of these biocatalysts under a more acidic pH.

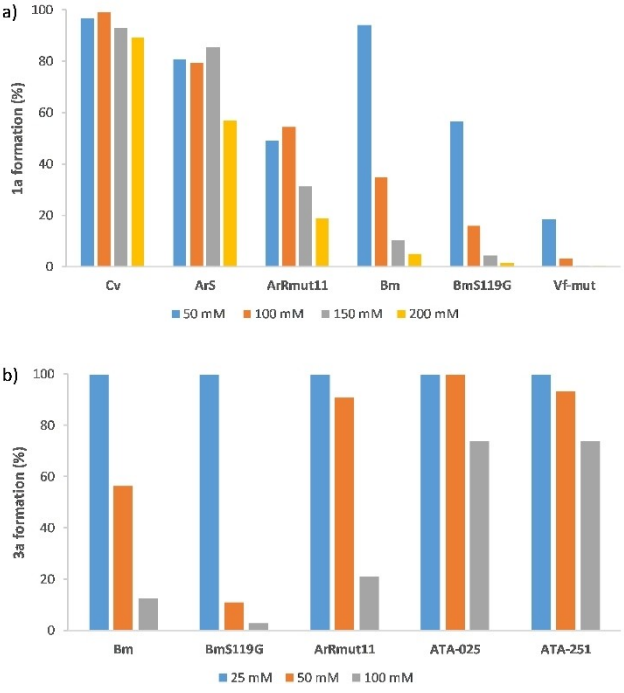


Figure 5. a) Transamination of furfural (**1 b**) at increasing concentrations of the substrate (50–200 mM) at pH 6.5. b) Transamination of DFF (**3 b**) at increasing concentrations of the substrate (25–100 mM) at pH 6.5. Conversion values were determined by GC and reverse phase HPLC analyses, respectively, of the crude reaction mixtures using calibration curves (see Section VI in SI).

Nevertheless, the impact of pH on the biotransamination of these furan-based aldehydes was evident from the results, emphasizing the need for careful investigation of this parame-

ter, particularly when using a highly nucleophilic amine donor such as isopropylamine.

Conclusions

The search for more sustainable alternatives to get access to bio-based products has become more appealing in recent years. Herein we have described the use of a series of amine transaminases to synthesize different furan-based amines with isopropylamine as cost-effective and easily accessible amine donors. Bio-based furfural, HMF, and DFF could be transformed into the final valuable amines under very mild conditions in aqueous medium by adding a molar excess of IPA. Among the different tested ATAs, overexpressed Cv-TA, ArS-TA, ArRmut11-TA, and Vf-mut-TA afforded very promising results in terms of conversion and selectivity, being able to produce the final products to high extent up to 200 mM of aldehyde concentration. Interestingly, it was observed that these transformations proceeded concurrently with the *in situ* formation of (poly)imines, due to the presence of reactive carbonyl species and the amines (IPA or amine products) in the medium. In the case of DFF, the formation of a solid residue was noticed, accounting for polymerization processes. Due to the reversibility of the imine bonds in aqueous media, for highly active ATAs it was possible to drive the equilibrium into the desired amine product. In fact, adjusting some parameters, such as pH, proved to be beneficial for the development of these amination processes. More acidic pHs hindered the imine formation, thereby improving the productivity of these biotransformations when enzyme stability and activity were not compromised.

Experimental Section

General

Materials: All chemicals were purchased from commercial sources. Commercially available aldehydes (furfural, HMF, and DFF) and amines (FAm, HMFA, and BAMF) were purchased from Sigma-Aldrich and used as standards. NMR spectra were recorded on a Bruker AV300 MHz spectrometer including ^{1}H and ^{13}C. All chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent signal. Gas chromatography (GC) analyses were performed on an Agilent HP6890 GC chromatograph equipped with a FID detector and DB-1701 column from Agilent (30 m×0.25 mm×0.25 μm). Analytical reverse phase analysis was performed using an Agilent 1100 Series HPLC equipped with a photodiode array detector, with a Mediterranea Sea C18 column from Teknokroma (18.5 μm×250 mm×4.6 mm). 10 μL of each sample were automatically injected. For biotransamination experiments with HMF, elution was carried out at 1 mL min^{-1} with a linear gradient of 15–75% of acetonitrile/H_2O containing 0.01% TFA over 30 min, with detection at 210 or 280 nm, and a column temperature of 30 °C. For biotransamination of DFF, elution was carried out at 0.9 mL min^{-1} with a linear gradient from 15% of acetonitrile to 100% (v/v, Section VI.2. in SI for details). The elution profiles of compounds were followed setting the detector at 210 and 280 nm, and a column temperature of 30 °C.

ATA cloning and expression

Cv-TA, ArR-TA, ArS-TA, ArRmut11-TA, Bm-TA, BmS199G-TA, Vf-mut-TA, Vf-TA, and At-TA were recombinantly expressed in *E. coli* BL21(DE3) cells (Invitrogen), using ampicillin or kanamycin as antibiotic. Detailed protocols for protein expression and cell lysis are provided in the Supporting Information file (Section II).

Measurement of ATA activities

The activity of the ATAs used as cell free extract (CFE) was determined with an adjusted standard photometric assay. Here, the substrate 1-phenylethylamine is converted to acetophenone, which can be detected spectrophotometrically at 245 nm. The absorbance increases with rising product concentration. To measure the activities, reactions were set up at 200 μL volume with 20 mM sodium phosphate buffer pH 8.0 containing 5 mM 1-phenylethylamine, and 5 mM sodium pyruvate. For the reaction, 100 μL of cell free lysates were used. The production of acetophenone was measured for 30 minutes at 245 nm. The volumetric activity as U mL^{-1} and the specific activity as U mg^{-1} total protein of the enzyme samples were calculated using the Beer-Lambert law with the molar extinction coefficient of acetophenone $\varepsilon_{245} = 12\ mM^{-1}\,cm^{-1}$.

The ATA activity of the lyophilized cell powders was assayed employing enantiopure (*S*)- or (*R*)-1-phenylethylamine as substrate (100 mM). Lyophilized *E. coli* cells (10 mg) were rehydrated in 1 mL of KPi buffer (100 mM, pH 7.0) containing PLP (1 mM) and sodium pyruvate (100 mM) for 30 min at 30 °C, 700 rpm. The substrate (100 mM, 12.1 μL) was then added (t = 0 min). The reactions were stopped after the set-up times (30 s, 60 s, 90 s, 120 s, 180 s, 240 s, and 300 s), and subjected to GC-FID analysis. The slope of the linear function that fitted the points collected during the first minutes was used to calculate the specific value of apparent enzymatic activity expressed as U mg^{-1} [μmol min^{-1} mg^{-1} cells)]. See Section II.6 in SI for further details.

General biotransamination experiments

Biotransamination of furfural. Reactions were performed on a total reaction volume of 1 mL, in an Eppendorf vial, containing the substrate (25–200 mM), DMSO (2.5%, v/v), PLP (1 mM), potassium phosphate buffer (100 mM, pH 7.5), IPA (1 M), and the corresponding *E. coli* lyophilized cells heterologously expressing the corresponding ATA (10 mg). The reaction mixture was incubated at 35 °C and 250 rpm for 24 h, and then stopped by the addition of an aqueous NaOH solution (10 M, 200 μL). The mixture was extracted twice with EtOAc (2×500 μL), and the organic layers were separated by centrifugation (2 min, 11,350 rpm). Later, they were combined and dried over Na_2SO_4. Degrees of conversion were determined by GC analyses (see Section VI.1 in SI).

Biotransamination of HMF. Reactions were performed on a total reaction volume of 500 μL in an Eppendorf vial, containing the substrate (25–200 mM), PLP (1 mM), potassium phosphate buffer (100 mM, pH 7.5), and the crude cell lysate (240 μL) or *E. coli* lyophilized cells heterologously expressing the corresponding ATA (5 mg). The concentration of IPA was 0.5 M, for enzymes used as cell free lysates, and 1 M for the enzymes used as *E. coli* lyophilized cells. After incubation at 30 °C and 250 rpm for 24 h, the reactions were stopped by the addition of 10% (v/v) trifluoroacetic acid (TFA) in water (25 μL). Denatured protein was removed by centrifugation (3,000 rpm, 4 min), and the supernatant diluted, filtered with a 45 μm micro-filter, and analysed by analytical HPLC. The substrate HMF was detected at 280 nm and the target amine HMFA at

210 nm. Conversions were obtained using calibration curves prepared with substrate and product standards (see Section VI.2 in SI).

Biotransamination of DFF. Reactions were performed on a total reaction volume of 1 mL in an Eppendorf vial, containing the substrate (25–200 mM), DMSO (2.5–5% v/v), PLP (1 mM), potassium phosphate buffer (pH 7.5, 100 mM) containing IPA (1 M), and the *E. coli* lyophilized cells heterologously expressing the corresponding ATA (10 mg) or a commercially available ATA (2 mg) from Codexis Inc. After incubation at 30 °C and 250 rpm for 24 h, samples were diluted with 500 μL of MeCN in order to solubilize all the compounds. Then, 20 or 25 μL of the crude sample were diluted in the HPLC mobile phase (MeCN/H_2O with TFA 0.01%, 85:15), according to the starting substrate concentration, filtered with a 45 μm micro-filter and injected in HPLC for the analysis (see Section VI.2 in SI).

Supporting Information

The SI contains full description of enzymes used in this contribution, enzyme screening studies, biotransamination optimization results, analytics, and NMR spectra for obtained compounds. The authors have cited additional references within the Supporting Information.[16a,18,29–33,37,38]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: amine transaminase · furan-derived amines · furfural · 5-(hydroxymethyl)furfural · isopropylamine

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