

Highly Efficient Oxidation of Amines to Aldehydes with Flow-based Biocatalysis

Martina L. Contente,^[a, b] Federica Dall'Oglio,^[c] Lucia Tamborini,^{*[c]} Francesco Molinari,^[d] and Francesca Paradisi^{*[a, b]}

1

A new mild and efficient process for the aqueous preparation of aldehydes, which are employed as flavour and fragrance components in food, beverage, cosmetics, as well as in pharmaceuticals, was developed using a continuous-flow approach based on an immobilised pure transaminase-packed bed reactor. HEWT, an ω -transaminase from the haloadapted bacterium *Halomonas elongata*, has been selected for its excellent stability and substrate scope. Sixteen different amines were rapidly (3–15 min) oxidised to the corresponding aldehydes (90 to 99%) with only 1 to 5 equivalents of sodium pyruvate. The process was fully automated, allowing for the in-line recovery of the pure aldehydes (chemical purity >99% and isolated yields above 80%), without any further work-up procedure.

Introduction

Aromatic aldehydes are important intermediates in a number of synthetic processes and have a prominent role as flavour and fragrance components. Among other synthetic methods,^[1] they can be obtained from the corresponding primary aromatic amines, which are readily available substrates. Methods for the oxidation of amines to carbonyl compounds have received significant attention, but these approaches are frequently poorly sustainable, because they produce waste and by-products that are difficult to recycle, require drastic reaction conditions, and often proceed with poor selectivity.^[1a, 2]

Biocatalytic processes are interesting alternatives for amine oxidations under mild and benign conditions. For example, copper amine oxidases (CAOs) have been used to catalyse the oxidation of primary amines to aldehydes (while O_2 is simultaneously reduced to H_2O_2).^[3] Vanillin has been prepared by oxidation of vanillylamine using an amine oxidase (AO) from *Aspergillus niger*.^[4] Recently, selective oxidation of amines to aldehydes has been obtained using a laccase with TEMPO (2,2,6,6-tetramethylpiperidine *N*-oxide) as mediator and O_2 as oxi-

[a]	Dr. M. L. Contente, Prof. F. Paradisi
	School of Chemistry
	University of Nottingham
	University Park, Nottingham, NG7 2RD (UK)
	E-mail: Francesca.Paradisi@nottingham.ac.uk

- [b] Dr. M. L. Contente, Prof. F. Paradisi UCD School of Chemistry University College Dublin Belfield, Dublin 4 (Ireland)
- [c] F. Dall'Oglio, Dr. L. Tamborini Department of Pharmaceutical Sciences, DISFARM University of Milan Via Mangiagalli 25, 20133 Milan (Italy) E-mail: lucia.tamborini@unimi.it
- [d] Prof. F. Molinari Department of Food, Environmental and Nutritional Science, DeFENS University of Milan via Mangiagalli 25, 20133 Milan (Italy)

dant.^[5] Aromatic aldehydes can also be enzymatically prepared using other approaches, such as oxidation of primary alcohols^[6] and reduction of carboxylic acids.^[7]

In this context, we developed an efficient bio-preparation of nature-identical flavours and fragrances exploiting the immobilised amine transaminase from the moderate halophilic bacterium *Halomonas elongata* (HEWT),^[8] which is able to tolerate a range of temperature, pH, salts and co-solvents in a continuous flow reactor. The combination of biocatalysis and flow reactor technology can be considered as an enabling methodology intrinsically compatible with the principles of green chemistry.^[9] Flow-based biocatalysis was recently applied for peptide condensation,^[10] hydrolysis and formation of esters and sugars,^[11] stereoselective carbonyl reduction,^[12] formation of C–C bonds,^[13] production of nucleosides,^[14] monosaccharides,^[15] and oligosaccharides,^[16] and interconversion of carbonyls and amines using transaminases.^[17]

We recently reported on the application of HEWT in flow for the biosynthesis of amines^[18] and we describe here an ecofriendly and scalable process that enhances the oxidising capability of this covalently immobilised enzyme for the production of aldehydes. The products are aromatic aldehydes used as flavours and fragrances in food, beverage, cosmetics and pharmaceuticals. They have been obtained in excellent yields, with unprecedented reaction times if compared with traditional batch methods. The use of pyruvate as amino acceptor is extremely favourable and by-product which it generates, the natural amino acid L-alanine, is completely benign and can be easily recovered. Furthermore, this approach circumvents potential issues often encountered with whole-cell biotransformations, such as generation of debris, swelling and permeability.

ChemCatChem 2017, 9, 1–7 Wiley Online Library



Results and Discussion

Pure HEWT (imm-HEWT) was immobilised on an epoxy-resin as reported by Planchestainer et al.^[18] and the supported biocatalyst (5 mg gram_{resin}⁻¹) was then used in a packed-bed flow reactor. The system was firstly tuned by optimising the preparation of benzaldehyde starting from the corresponding benzylamine (Scheme 1).



Scheme 1. Solution A: 20 mM solution of benzylamine in phosphate buffer (50 mM, pH 8.0) containing 10% DMSO. Solution B: 20 mM solution of pyruvate containing 0.1 mM PLP. T=37 °C, P=atm.

To maximise the solubility of the amine, 10% of DMSO was used as co-solvent in the phosphate buffer (50 mm, pH 8.0). The reaction was performed under optimised conditions at 37°C and atmospheric pressure with just one equivalent of pyruvate, as the equilibrium for this reaction is extremely favourable; complete substrate oxidation (molar conversion > 99%) was obtained with only 3 minutes of residence time (flow rate 0.3 mLmin⁻¹).

Notably, the use of the same immobilised enzyme in batch gave a full oxidation in about 2 hours.

The optimised conditions were applied to the bioconversion of different benzylamines into the corresponding flavour aldehydes (Table 1).

Specific reaction rates in the batch and continuous-flow systems were calculated using the equations reported in the Experimental Section; the time taken (conversion rate) for the reaction to reach maximum conversion, whether in batch or continuous-flow, was calculated and normalised to the amount of catalyst used for both set-ups.^[11a]

Benzylamine-derivatives (entries 1–8) were oxidised into the corresponding aroma-compounds with high molar conversion; in all cases, a greater than 4-fold rate increase was observed if reactions were conducted under flow conditions, as conversions \geq 90% were reached within a residence time between 3 and 10 minutes (flow rate 0.3 mLmin⁻¹ and 0.1 mLmin⁻¹, respectively), at 37 °C and atmospheric pressure.

The process was implemented with the addition of an inline acidification step followed by extraction with EtOAc. The two phases were continuously separated using a Zaiput liquid/ liquid separator and the desired aldehydes were recovered in the organic phase, significantly accelerating the overall workup, as no further purification is required (Scheme 2).

This protocol was successfully applied to substrates **1a-1h**. Aldehydes obtained from substrates **1i** and **1j** (entries 9 and 10) proved initially difficult to recover as they were retained by





Scheme 2. Solution A: 20 mM solution of amines (entries 1–8) in phosphate buffer (50 mM, pH 8.0) with 10% DMSO. Solution B: 20 mM, solution of pyruvate containing 0.1 mM PLP. T=37 °C, P=atm.

the packing material, despite various and extensive washing steps.

A liquid-liquid-phase reaction system was therefore set up, in which toluene flowed into the system upstream of the packed column (Scheme 3). On acidification, downstream of the process, the products **2i** and **2j** were extracted in-line and recovered by membrane separation as pure compounds. Remarkably, the presence of toluene had no effect on the catalytic efficiency of the immobilised enzyme which was extensively used over several weeks.



Scheme 3. Solution A: 20 mM solution of amines (entries 9, 10 Table 1 or 14–16 Table 2) in phosphate buffer (50 mM, pH 8.0). Solution B: 20 mM, 40 mM or 100 mM solution of pyruvate containing 0.1 mM PLP. T=37 or 45 °C, P= atm. Toluene is added at the same flow rate to form a 50:50 biphasic stream.

A second set of amines (1 k-1 p) was investigated using the same methodologies (either in a monophasic environment or the biphasic one) to prove the versatility of the system with different aromatic substrates. (Table 2).

The batch oxidation of the tested (aryl)alkyl amines with methyl/ethyl side chain (entries 11–14) allowed for the preparation of flavour aldehydes 2k (hyacinth note), 2j (floral note), 2m (floral note), and 2n (violet note) with excellent conversion (>99%), although the reactions required several hours to go to completion. In line with our observations for the benzylamine derivatives, the same molar conversion was obtained within 3 to 15 minutes of residence time in flow, thus strongly increasing the overall productivity. In particular, piperonylamine (1 n) was successfully converted into the corresponding aldehyde (piperonal 2n, the violet fragrance, also known as heliotropin) in only 15 minutes (14-fold faster reaction rate) with >99% conversion at 45°C, demonstrating the good stability and adaptability of this enzyme also at higher temperatures. Both (*S*) and (*R*)-2-phenyl-1-propylamine (11 and 1 m, respec-



CHEMCATCHEM Full Papers

Table 1. Preparation of aromatic benzaldehyde derivatives from the corresponding amines. ^[a] R I NH2 immobilized HEWT/PLP CHO Phosphate buffer (50 mM, pH 8.0) R I CHO									
		1	о /	NH₂ → OH	2				
Entry	Substrate	Reaction time [min]	M. c. [%]	Conv. Rate ^[b] [μ mol min ⁻¹ g ⁻¹]	Residence time [min]	M. c. [%]	Conv. Rate ^(b) $[\mu mol min^{-1}g^{-1}]$		
1	NH ₂ 1a	120	>99	0.83	3	>99	4.24		
2	NH ₂ 1b	120	>99	0.83	3	>99	4.24		
3	MeO 1c	120	>99	0.83	3	>99	4.24		
4	1d	120	>99	0.83	3	>99	4.24		
5	HO 1e	120	>99	0.33	10	>99	1.41		
6	NH ₂ 1f	300	>99	0.33	10	90	1.29		
7	OH 1g	300	>99	0.33	10	90	1.29		
8	HO HO 1h	120	>99	0.83	3	95	4.07		
9	MeO MeO 1i	300	>99	0.33	10	>99 ^[c]	1.41 ^[c]		
10	HO HO 1j	300	> 99	0.33	10	>99 ^(c)	1.41 ^[c]		

[a] Reactions were performed in the presence of 10 mM substrates and pyruvate, 0.1 mM PLP, 10% DMSO was used as co-solvent at 37 °C. Isolated yields are reported in the Experimental Section. [b] Conversion rates are normalised to the amount of enzyme used in the reaction and calculated as reported in Ref. [11a]. [c] Liquid-liquid-phase flow stream (see procedure summarised in Scheme 3), in this case DMSO was not added to the buffer.

tively) were suitable substrates for HEWT. The enzyme equally converted both enantiomers and did not show any stereopreference for this particular molecule (entries 12 and 13).

However, the oxidation of cinnamylamine (**1o**, entry 15) to cinnamaldehyde (**2o**, cinnamon aroma) and hydrocinnamylamine (**1p**, entry 16) to hydrocinnamaldehyde (**2p**, honey aroma), appeared more challenging. The batch reaction with an equimolar concentration of amino donor resulted in poor

conversion after 24 hours (50 and 52%), without any significant increase over a longer incubation time, likely owing to an unfavourable equilibrium. Under flow conditions, with one equivalent of pyruvate, the conversions achieved were 50% and 25% respectively, despite increasing the residence time to 30 min. To displace the equilibrium, the concentration of pyruvate was increased to 2 and 5 equivalents with respect to the aldehydes **1o** and **1p**, yielding 95% of cinnamaldehyde and

3



Table 2. Preparation of aryl-alkyl aldehydes from the corresponding amines. ^[a]									
Entry	Substrate	Reaction time [min]	M.c. [%]	Conv. Rate ^[b] [μ mol min ⁻¹ g ⁻¹]	Residence time [min]	M.c. [%]	Conv. Rate ^(b) [μ mol min ⁻¹ g ⁻¹]		
11	NH ₂	120	>99	0.83	3	> 99	4.24		
12		180	> 99	0.55	3	> 99	4.24		
13	NH ₂	180	>99	0.55	3	> 99	4.24		
14		1440	>99	0.07	15	$> 99^{[c,d]}$	0.95 ^[c,d]		
15		1440	50	0.04	15	> 99 ^[c,d,e]	1.02 ^(c,d,e,g)		
16	1p	300	24	0.04	15	90 ^[c,d,f]	0.86 ^[c,d,f,g]		

[a] Reactions were performed in the presence of 10 mM substrates and pyruvate, 0.1 mM PLP, 10% DMSO was used as co-solvent at 37 °C. Isolated yields are reported in the Experimental Section. [b] Conversion rates are normalised to the amount of enzyme used in the reaction and calculated as reported in Ref. [11a]. [c] Liquid-liquid-phase flow stream (see procedure summarised in Scheme 3), in this case DMSO was not added to the buffer. [d] Reactions performed at 45 °C. [e] 20 mM Pyruvate. [g] 50 mM pyruvate. [g] Calculated at a similar degree of conversion of the batch reaction.

90% of the saturated aldehyde with 15 minutes of residence time at 45 °C. This result underlines the fact that process control strategies (in our case, the optimisation of stoichiometric ratio of the substrates) help to maximise the productivity of HEWT by accelerating the reaction, while shifting the equilibrium to the product's side.

Conclusions

A new biocatalytic method for the synthesis of aldehydes with extensive applications as components of flavours and fragrances was developed. This is the first example of a transaminase exploited in a flow chemistry reactor under highly favourable oxidising conditions for the preparation of aromatic aldehydes, showing excellent adaptability and stability during the processes. The use of a flow-based approach allowed for dramatic accelerations of the reactions, with isolated yields above 80% and very short residence times (3-15 min) of the substrates. This system required, in the majority of cases, only one equivalent of pyruvate as the amino acceptor, which leads to the formation of L-alanine as by-product. A successful implementation was achieved with an in-line extraction step, which permitted the recovery of the desired pure aldehydes in the organic stream and L-alanine in the aqueous one, with an extremely simplified work-up procedure and almost no manipulation. As a result of the high local concentration of the (bio)catalyst and the enhanced heat and mass transfer,^[19] the combination between biocatalysis and flow chemistry reactors not only leads to significant reductions of reaction times and increased productivity, but it can be also considered a sustainable technology for the production of aldehydes commonly used in food, cosmetic, and pharmaceutical industry.

Experimental Section

Expression, purification, and immobilisation of HEWT in *E. coli*

Protein expression and purification was performed following previously reported protocols in Cerioli et al.,^[8] immobilisation was conducted according to the procedure reported by Planchestainer et al.^[18]

Batch reactions with immobilised HEWT

Batch reactions using the imm-HEWT were performed in 1.5 mL micro centrifuge tubes; 500 μ L reaction mixture in 50 mm phosphate buffer pH 8.0, containing 10 mm pyruvate, 10 mm amino donor substrate, 0.1 mm PLP, and 50 mg of imm-HEWT (5 mg g⁻¹) was left under gentile shaking at 37 °C. 10 μ L aliquots were quenched with trifluoroacetic acid (TFA) 0.2% every hour and then analysed by HPLC equipped with a Supelcosil LC-18-T column (250 mm×4.6 mm, 5 μ m particle size; Supelco, Sigma–Aldrich, Ger-

www.chemcatchem.org



many). The compounds were detected using an UV detector at 210 nm, 250 nm or 280 nm after an isocratic run with 25% acetonitrile/75% water with TFA 0.1% v/v at 25°C with a flow rate of 1 mLmin⁻¹. The retention times in minutes are: benzylamine (4.1 min), benzaldehyde (9.4 min), p-methylbenzylamine (5.2 min), p-tolualdehyde (16.4 min), p-methoxybenzylamine (4.4 min), p-anisaldehyde (10.3 min), p-ethylbenzylamine (5.0 min), p-ethylbenzaldehyde (16.5 min), p-hydroxybenzylamine (3.8 min), p-hydroxybenzaldehyde (10.5 min), p-isopropylbenzylamine (10.0 min), cuminaldehyde (35.0 min), 2-(aminomethyl)-phenol (3.7 min), salicylaldehyde (10.3 min), vanillylamine (3.7 min), vanillin (5.7 min), veratrylamine (4.1 min), veratraldehyde (8.0 min), 4-(aminomethyl)-2,6dimethoxyphenol (3.5 min), syringaldehyde (5.4 min), 2-phenethylamine (3.9 min), phenylacetaldehyde (9.8 min), (R)-2-phenyl-1-propylamine (4.3 min), (S)-2-phenyl-1-propylamine (4.3 min), 2-phenylpropanaldehyde (10.9 min), piperonylamine (4.2 min), piperonal (9.9 min), cinnamylamine (6.6 min), cinnamaldehyde (15.6 min), hydrocinnamylamine (5.1 min), hydrocinnamaldehyde (13.6 min), confirmed by comparison with commercially available compounds.

Flow reactions with immobilised HEWT

Continuous flow biotransformations were performed using a R2+ /R4 Vapourtec flow reactor equipped with an Omnifit glass column (0.3421 mm i.d \times 100 mm length) filled with 0.7 g of imm-HEWT (5 mg g⁻¹). A 20 mм sodium pyruvate in phosphate buffer (50 mм, pH 8.0) containing 0.1 mм pyridoxal phosphate, and 20 mм amino donor solution with 10% of DMSO were prepared. The two solutions were mixed in a T-piece and the resulting flow stream was directed into the column packed with the biocatalyst (packed bed reactor volume: 1.0 mL). The flow rate was varied and optimised. An in-line acidification was performed by using an inlet of 1 N HCl aqueous solution (flow rate: 0.1 mLmin⁻¹) that was mixed to the exiting reaction flow stream using a T-junction. The resulting aqueous phase was extracted in-line using a stream of EtOAc (flow rate: 0.2 mLmin⁻¹) and a Zaiput liquid/liquid separator. Both the organic and aqueous phase were analysed by HPLC using the above reported conditions. The amount of substrate and product was evaluated by exploiting a previously prepared calibration curve. For the optimisation procedure, the reactions have been performed by injecting 250 μ L of each starting solutions (volume of EtOAc used for the in-line extraction: 1 mL). To isolate the product, 10 mL of each starting solution has been used (volume of EtOAc used for the in-line extraction: 40 mL). The organic phase, containing the aldehyde, was evaporated to yield the desired product.

Specific reaction rates in batch and continuous-flow systems were calculated using Equations 1 and 2:

$$r_{\text{batch}} = \frac{\eta_{\text{P}}}{t \times m_{\text{B}}} (\mu \text{mol} / \min g)$$
(1)

where $[n_p]$ is the amount of product (expressed in µmol), *t* is the reaction time (expressed in min), and m_B [g] is the amount of biocatalyst employed.

$$r_{\rm flow} = \frac{[P] \times f}{m_{\rm B}} (\mu {\rm mol}/\min g)$$
⁽²⁾

where [P] is the product concentration flowing out of the reactor (expressed in μ mol mL⁻¹), f is the flow rate (expressed in mL min⁻¹), and m_{B} [g] is the amount of biocatalyst loaded in the column.

Comparison of the rates of the same reaction in a batch or flowmode was made at similar degrees of conversion.

Flow reactions in liquid-liquid-phase systems with immobilised HEWT

20, 40 or 100 mM pyruvate in phosphate buffer (50 mM, pH 8.0) containing 0.1 mM PLP, and 20 mM amino donor solutions were prepared. The two solutions were mixed in a T-piece. A second junction for additional supplement of toluene at the same flow rate was installed before the packed enzyme column. The resulting segmented flow stream was directed to the imm-HEWT. The flow rate was varied and optimised. After an in-line acidification step, as previously reported, the exiting flow stream was separated by a Zaiput liquid/liquid separator. The organic and aqueous phases were analysed by HPLC, exploiting a calibration curve (see conditions above), and the toluene containing the desired product was evaporated to yield the aldehydes.

Characterisation of the products

The purity of aldehydes was assessed by HPLC and ¹H NMR. ¹H NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling constants (*J*) are expressed in Hz.

Benzaldehyde (2a): colourless oil; yield 95%; ¹H NMR (CDCl₃) δ = 10.00 (s, 1H), 8.15–8.12 (m, 2H), 7.67–7.51 ppm (m, 3H).

p-Tolualdehyde (2b): yellow oil; yield 96%; ¹H NMR (CDCI₃) δ = 9.95 (s, 1 H), 7.74 (d, J=7.5 Hz, 2 H), 7.32 (d, J=7.5 Hz, 2 H), 2.40 ppm (s, 3 H).

p-Anisaldehyde (2 c): colourless oil; yield 94%; ¹H NMR (CDCl₃) δ = 9.85 (s, 1 H), 7.80 (d, J=8.0 Hz, 2 H), 6.96 (d, J=8.0 Hz, 2 H), 3.90 ppm (s, 3 H).

*p***-Ethyl benzaldehyde (2 d)**: yellow oil; yield 94%; ¹H NMR (CDCl₃) δ = 9.98 (s, 1 H), 7.81 (d, *J* = 8.1 Hz, 2 H), 7.36 (d, *J* = 8.1 Hz, 2 H), 2.74 (q, *J* = 7.5 Hz, 2 H), 1.27 ppm (t, *J* = 7.5 Hz, 3 H).

*p***-Hydroxybenzaldehyde (2 e)**: yellow solid; yield 92%; ¹H NMR (CDCl₃): δ =9.61 (s, 1 H), 7.60 (d, J=8.3 Hz, 2 H), 6.73 ppm (d, J=8.3 Hz, 2 H).

Cuminaldehyde (2 f): colourless oil; yield 84%; ¹H NMR (CDCl₃) δ = 9.98 (s, 1 H), 7.84 (d, *J*=8.4 Hz, 2 H), 7.40 (d, *J*=8.4 Hz, 2 H), 3.00 (septet, *J*=6.9 Hz, 1 H), 1.30 ppm (d, *J*=6.9 Hz, 6 H).

Salicylaldehyde (2g): yellow oil; yield 82%; ¹H NMR (CDCl₃) $\delta\!=$ 11.00 (bs, 1H, OH), 9.85 (s, 1H), 7.46–7.54 (m, 2H), 6.94–7.00 ppm (m, 2H).

Vanillin (2 h): white solid; yield 90%; ¹H NMR (CDCl₃) δ =9.78 (s, 1 H), 7.37–7.40 (m, 2 H), 7.02 (d, *J*=8.5 Hz, 1 H), 6.72 (bs, 1 H, OH), 3.90 ppm (s, 3 H).

Veratrylaldehyde (2 i): yellow solid; yield 96 %; ¹H NMR (CDCl₃) δ = 9.85 (s, 1 H), 6.70–7.65 (m, 3 H), 3.98 (s, 3 H), 3.95 ppm (s, 3 H).

Syringaldehyde (2j): yellow solid; yield 94%; ¹H NMR (CDCl₃) δ = 9.83 (s, 1 H), 7.15 (s, 2 H), 6.10 (s, 1 H), 3.98 ppm (s, 6 H).

ChemCatChem 2017, 9, 1–7 www.chemcatchem.org

These are not the final page numbers! **77**



Phenylacetaldehyde (2 k): pale yellow oil; yield 97%; ¹H NMR (CDCl₃) δ =9.70 (t, J=2.0 Hz, 1 H), 7.30–7.10 (m, 5 H), 3.56 ppm (d, J=2.0 Hz, 2 H).

2-Phenylpropanaldehyde (21/2 m): colourless oil; yield 90%; ¹H NMR (CDCl₃) δ =9.62 (s, 1H), 7.30–7.40 (m, 2H), 7.20–7.28 (m, 3H), 3.60 (q, *J*=7.0, 1H), 1.45 ppm (d, *J*=7.0, 3H).

Piperonal (2 n): white solid; yield 87%; ¹H NMR (CDCl₃) δ = 9.80 (s, 1 H), 7.40 (dd, *J*=7.9, 1.6 Hz, 1 H), 7.32 (d, *J*=1.6 Hz, 1 H), 6.92 (d, *J*=7.9 Hz, 1 H), 6.07 ppm (s, 2 H).

Trans-Cinnamaldehyde (2 o): yellow oil; yield 89% ¹H NMR (CDCl₃) δ = 9.70 (d, J=7.7 Hz 1 H), 7.55 (dd, J=5.2, 2.0 Hz, 2 H), 7.50 (d, J= 15.9 Hz, 1 H), 7.42–7.46 (m, 3 H), 6.73 ppm (dd, J=15.9, 7.7 Hz, 1 H).

Hydrocinnamaldehyde (2 p): pale yellow oil: yield 86% ¹H NMR (CDCl₃) δ =9.76 (s, 1 H), 7.35 (q, *J*=7.4 Hz, 2 H), 7.25–7.30 (m, 3 H), 3.00 (t, *J*=15.1 Hz, 2 H), 2.82–2.85 ppm (m, 2 H).

Acknowledgements

The authors wish to thank UK Biotechnology and Biological Sciences Research Council (BBSRC; BB/P002536/1) for financial support (F.P., M.L.C), Science Foundation Ireland for initial funding through Synthesis and Solid State Pharmaceutical Centre code 12/RC/2275 (reagents and support, F.P).

Conflict of interest

The authors declare no conflict of interest.

Keywords: Aldehydes · Amine oxidation · Biocatalysis · Flow reactor · Transaminase

- a) W. Kantlehner, *Eur. J. Org. Chem.* 2003, 2530–2546; b) "Preparation of Aldehydes": M. B. Smith in *Compendium of Organic Synthetic Methods, Vol. 13*, Wiley-VCH, Weinheim, 2014, pp. 75–88.
- [2] a) T. F. Buckley, H. Rapoport, J. Am. Chem. Soc. 1982, 104, 4446-4450; b) Y. Hu, H. Hu, Synth. Commun. 1992, 22, 1491-1496; c) K. Orito, T. Hatakeyama, M. Takeo, S. Uchiito, M. Tokuda, H. Suginome, Tetrahedron 1998, 54, 8403-8410; d) N. A. Noureldin, J. W. Bellegarde, Synthesis 1999, 939-942; e) J. Matsuo, A. Kawana, Y. Fukuda, T. Mukaiyama, Chem. Lett. 2001, 30, 712-713; f) S. Sharma, N. Barooah, J. B. Baruah, J. Mol. Catal. A 2005, 229, 171-176; g) H. Hamamoto, Y. Suzuki, H. Takahashi, S. Ikegami, Tetrahedron Lett. 2007, 48, 4239-4242; h) D. A. Knowles, C. J. Mathews, N. C. O. Tomkinson, Synlett 2008, 2769-2772; i) L. Liu, S. Zhang, X. Fu, C. H. Yan, Chem. Commun. 2011, 47, 10148-10150; i) S. Desjardins, G. Jacquemot, S. Canesi, Synlett 2012, 23, 1497-1500; k) M. T. Schümperli, C. Hammond, I. Hermans, ACS Catal. 2012, 2, 1108-1117; I) S. E. Allen, R. R. Walvoord, R. Padilla-Salinas, M. C. Kozlowski, Chem. Rev. 2013, 113, 6234-6458; m) M. Largeron, Eur. J. Org. Chem. 2013, 5225-5235; n) M. Largeron, Org. Biomol. Chem. 2017, 15, 4722-4730.
- [3] a) A. Hacisalihoglu, A. Jongejan, J. A. Jongejan, J. A. Duine, J. Mol. Catal.
 B 2000, 11, 81-88; b) N. J. Turner, Chem. Rev. 2011, 111, 4073-4087;

c) E. I. Solomon, D. E. Heppner, E. M. Johnston, J. W. Ginsbach, J. Cirera, M. Qayyum, M. T. Kieber-Emmons, C. H. Kjaergaard, R. G. Hadt, L. Tian, *Chem. Rev.* **2014**, *114*, 3659–3853.

- [4] A. Yoshida, Y. Takenaka, H. Tamaki, I. Frébort, O. Adachi, H. Kumagai, J. Ferment. Bioeng. 1997, 84, 603–605.
- [5] P. Galletti, F. Funiciello, R. Soldati, D. Giacomini, Adv. Synth. Catal. 2015, 357, 1840–1848.
- [6] a) F. Molinari, *Curr. Org. Chem.* 2006, *10*, 1247–1263; b) D. Romano, R. Villa, F. Molinari, *ChemCatChem* 2012, *4*, 739–749; c) P. Zambelli, A. Pinto, D. Romano, E. Crotti, P. Conti, L. Tamborini, R. Villa, F. Molinari, *Green Chem.* 2012, *14*, 2158–2161; d) A. Díaz-Rodríguez, L. Martínez-Montero, I. Lavandera, V. Gotor, V. Gotor-Fernández, *Adv. Synth. Catal.* 2014, *356*, 2321–2329; e) R. A. Sheldon, *Catal. Today* 2015, *247*, 4–13; f) Y.-G. Zheng, H.-H. Yin, D.-F. Yu, X. Chen, X.-L. Tang, X.-J. Zhang, Y.-P. Xue, Y.-J. Wang, Z.-Q. Liu, *Appl. Microbiol. Biotechnol.* 2017, *101*, 987–1001.
- [7] a) M.-K. Akhtar, N. J. Turner, R. Patrik, P. R. Jones, *Proc. Natl. Acad. Sci.* USA 2013, 110, 87–92; b) A. M. Kunjapur, Y. Tarasova, K. L. J. Prather, *J. Am. Chem. Soc.* 2014, 136, 11644–11654; c) W. Finnigan, A. Thomas, H. Cromar, B. Gough, R. Snajdrova, J. P. Adams, J. A. Littlechild, N. J. Harmer, *ChemCatChem* 2017, 9, 1005–1017.
- [8] L. Cerioli, M. Planchestainer, J. Cassidy, D. Tessaro, F. Paradisi, J. Mol. Catal. B 2015, 120, 141-150.
- [9] a) S. V. Ley, Chem. Rec. 2012, 12, 378–390; b) J. M. Bolivar, J. Wiesbauer,
 B. Nidetzky, Trends Biotechnol. 2011, 29, 333–342.
- [10] P. Falus, L. Cerioli, G. Bajnoczi, Z. Boros, D. Weiser, J. Nagy, D. Tessaro, S. Servi, L. Poppe, Adv. Synth. Catal. 2016, 358, 1608 1617.
- [11] a) C. Csajági, G. Szatzker, E. R. Toke, L. Ürge, F. Darvasa, L. Poppe, *Tetrahedron: Asymmetry* **2008**, *19*, 237–246; b) I. I. Junior, M. C. Flores, F. K. Sutili, S. G. F. Leite, L. S. D. M. Miranda, I. C. R. Leal, R. O. M. A. de Souza, *Org. Process Res. Dev.* **2012**, *16*, 1098–1101; c) L. Tamborini, D. Romano, A. Pinto, A. Bertolani, F. Molinari, P. Conti, *J. Mol. Catal. B* **2012**, *84*, 78–82; d) L. Tamborini, D. Romano, A. Pinto, M. Contente, M. C. Iannuzzi, P. Conti, F. Molinari, Tetrahedron Lett. **2013**, *54*, 6090–6093; e) I. Itabaian, L. S. de Miranda, R. O. M. A. de Souza, *J. Mol. Catal. B* **2013**, *85–86*, 1–9; f) S. S. Wang, Z. J. Li, S. Sheng, F. A. Wu, J. Wang, *J. Chem. Technol. Biotechnol.* **2016**, *91*, 555–562; g) F. Carvalho, M. P. C. Marques, P. Fernandes, *Catalysts* **2017**, *7*, 42–60.
- [12] F. Dall'Oglio, M. L. Contente, P. Conti, F. Molinari, D. Monfredi, A. Pinto, D. Romano, D. Ubiali, L. Tamborini, I. Serra, *Catal. Commun.* 2017, *93*, 29–32.
- [13] J. Lawrence, B. O'Sullivan, G. J. Lye, R. Wohlgemuth, N. Szita, J. Mol. Catal. B 2013, 95, 111–117.
- [14] E. Calleri, G. Cattaneo, M. Rabuffetti, I. Serra, T. Bavaro, G. Massolini, G. Speranza, D. Ubiali, Adv. Synth. Catal. 2015, 357, 2520–2528.
- [15] L. Babich, A. F. Hartog, L. J. C. van Hemert, F. P. J. T. Rutjes, R. Wever, *ChemSusChem* 2012, *5*, 2348–2353.
- [16] P. Zambelli, L. Tamborini, S. Cazzamalli, A. Pinto, S. Arioli, S. Balzaretti, F. J. Plou, L. Fernandez-Arrojo, F. Molinari, P. Conti, D. Romano, *Food Chem.* 2016, 190, 607–613.
- [17] a) L. H. Andrade, W. Kroutil, T. F. Jamison, Org. Lett. 2014, 16, 6092–6095; b) M. Bajic, I. Plazl, R. Stloukal, P. Znidarsic-Plazl, Process Biochem. 2017, 52, 63–72.
- [18] M. Planchestainer, M. L. Contente, J. Cassidy, F. Molinari, L. Tamborini, F. Paradisi, *Green Chem.* 2017, 19, 372–375.
- [19] a) C. Wiles, P. Watts, *Green Chem.* 2012, *14*, 38–54; b) P. Plouffe, A. Macchi, D. M. Roberge, *Org. Process Res. Dev.* 2014, *18*, 1286–1294; c) M. C. Bryan, B. Dillon, L. G. Hamann, G. J. Hughes, M. E. Kopach, E. A. Peterson, M. Pourashraf, I. Raheem, P. Richardson, D. Richter, H. F. Sneddon, *J. Med. Chem.* 2013, *56*, 6007–6021.

Manuscript received: July 14, 2017

Version of record online:

FULL PAPERS

Going with the flow: A new continuous-flow biocatalytic process for the oxidation of amines to aldehydes, commonly used as flavours and fragrances, was achieved in an enzyme-mediated single step. An ω -transaminase from *Halomonas elongata* was exploited as a heterogeneous biocatalyst in a packedbed reactor, under highly favourable reaction conditions in the presence of pyruvate, generating L-alanine as a benign by-product.



M. L. Contente, F. Dall'Oglio, L. Tamborini,* F. Molinari, F. Paradisi*



Highly Efficient Oxidation of Amines to Aldehydes with Flow-based Biocatalysis