Micro-scale Methods for Rapid Evaluation of Process Options too Increase the Yield of Equilibrium-Controlled Bioconversions: Application to the ω-Transaminase Synthesis of Chiral Amines‡.

Murni Halim[1], Leonardo Rios - Solis[1], Frank Baganz[1], Martina Micheletti[1], John M. Ward[2], Gary J. Lye[1+]

[1] Department of Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE, UK

[2]Institute of Structural and Molecular Biology, University College London, Gower Street, London, WC1E 6BT, UK

Keywords: ω -transaminase, asymmetric synthesis, microscale, equilibrium-controlled reaction, product inhibition

‡ Manuscript prepared for Biocatalysis and Biotransformation.

+Corresponding author (Email: g.lye@ucl.ac.uk)

ABSTRACT

As an example for rapid evaluation of process options for overcoming low yielding equilibrium-controlled reactions, an asymmetric synthesis of the 2−amino−1,3,4−butanetriol (ABT) using prochiral ketone L-erythrulose (Ery) was carried out with ω-transaminase from *Chromobacterium violaceum* DSM30191 (CV2025 TAm). Four process options to increase reaction yield at 50mM amino donor substrate S-α-methylbenzylamine (MBA) which has typical yield of just 26% (w/w) ABT, have been developed by overcoming the inhibition of by-product acetophenone (AP). The first option is examining CV2025 TAm bioconversion with different type of amino donors as the alternative for MBA. The second option couples CV2025 TAm with alcohol dehydrogenase (ADH) and glucose dehydrogenase (GDH) for removing the inhibitory AP by conversion to 1-phenylethanol. The final approaches are physical in *situ* product removal (ISPR) methods, applying reduced pressure conditions (i.e., 5 torr) for evaporation of the volatile AP and utilizing hydrophobic polymer resin for adsoption of AP in order to eliminate it from the reaction system. An excess of amino acceptor substrate drives the reaction conversion much higher than in a reaction having a similar molar ratio of amino donor and amino acceptor helps to significantly accelerate the initial rate of the reaction. All systems can be performed in microwell experiments capable of automation platform and increases in product yield with exception only for adsorbent resins system. The best result obtained with the second option yielded 98% (w/w) ABT followed by the first option when MBA was replaced with Isopropylamine (IPA) and reached 72% (w/w) ABT.

1. INTRODUCTION

Recently, transaminas have received increased interest due to their great potential for the production of natural and non-natural amino acids as well as other chiral amines, which are in demand by the pharmaceutical industry (Christen and Metzler, 1985; Sheldon, 1996; Shin and Kim, 1997; Stirling, 1992; Shin and Kim, 2001). Optically active amines are required for the preparation of a broad range of biologically active compounds showing various pharmacological properties (Sutin, 2007; Dominik *et al*., 2008) such as building blocks in the synthesis of neurological, immunological, anti-hypertensive and anti-infective drugs. At present, it is estimated that approximately 75% of pharmaceuticals on the market involve insertion or modification of an amino group during their synthesis. Enantioenriched amines can be produced by either asymmetric synthesis (Shin and Kim, 1999) using a chiral ketone (i.e amination of the ketone) or kinetic resolution (Shin and Kim, 1997) of racemic amine (i.e deamination of the amine).

The ω-Transaminase from *Chromobacterium violaceum* DSM30191 (CV2025 TAm) has been recently cloned into the expression vector pET29a and expressed in *Escherichia coli (E.coli)* BL21 (Kaulmann *et al*., 2007). CV2025 TAm shows high enantioselectivity for (S)-enantiomer of various amines compounds such as α-methylbenzylamine (MBA). This high stereo selectivity could lead to a useful asymmetric strategy for the preparation of enantiopure amines and amino alcohols (Shin *et al*., 2001; Yun *et al*., 2003; Yun *et al*., 2005; Kaulmann *et al*., 2007). The asymmetric synthesis of chiral amines with ω-transaminases may offer many potential advantages over kinetic resolution, such as the lack of need for reductive amination to prepare racemic amine from ketone precursor, chiral precursors are

usually less expensive than racemic mixtures (Sheldon, 1996; Shin and Kim, 1999) and twofold higher theoretical yield (Shin and Kim, 1999).

However, the challenges of using this method is to shift the equilibrium to product formation, especially when using amino acid as an amino donor, stereoselectivity of the enzyme has to be perfect, which is not always the case for ω-TAm (Dominik K, *et al*., 2008) and much more severe product inhibition than kinetic resolution method (Shin and Kim, 1998; Shin and Kim, 1999). The product inhibition results from the left formation of Michaelis complex between product and enzyme. Due to the reversibility of the TAm reaction, products can bind to the enzyme and reduce the chances for substrates occupy an active site of the enzyme. The formation of the Michaelis complex commences a reverse reaction to occur at the same time and consequently reduce the net reaction rate. Additionally, a choice of amino donor is of great importance in the asymmetric reaction system. For instance, it is impractical to use some chiral amines as amino donor in synthesizing other chiral amines due to their high cost despite their high reactivity.

Therefore, the main goal of this research is to demonstrate process options of CV2025-TAm bioconversion by overcoming low equilibrium constant and inhibition of product in micro-scale reaction. The potential process options to overcome low yielding equilibrium-controlled reactions or those with inhibitory products are summarized in Figure 1. The micro-scale processing technique are rapidly emerging as a means to increase the speed of bioprocess design (Lye G.J, *et al*, 2003) and perhaps will enable a wider range of process variable to be examined in near future for an efficient industrial transamination process.

2. MATERIALS AND METHODS

2.1 MATERIALS

Nutrient broth and nutrient agar were obtained from Fisher Scientific (Leicestershire, UK). Transaminase expressions were obtained using plasmids pQR801 transformed into *E.coli* BL21 (DE3), which contains the complete *Chromobacterium violaceum* DSM30191 transaminase gene (Kaulmann et al., 2007). These strains were stored as glycerol stocks at -80°C. All other reagents were obtained from Sigma-Aldrich (Gillingham, UK) unless otherwise stated.

2.2 ENZYME PREPARATIONS

CV2025 TAm: For expression of ω-transaminase, 10% (v/v) inoculum of liquid overnight culture of *E.coli* BL21 (DE3)-pQR801, was inoculated into LB-Glycerol (LB-G) medium ($10g.L^{-1}$ tryptone, $5g.L^{-1}$ yeast extract, $10g.L^{-1}$ NaCl, $10g.L^{-1}$ glycerol) containing appropriate resistance antibiotic ($150\mu g.mL^{-1}$ kanamycin). Growth was performed at 37°C with orbital shaking at 250 rpm using an SI 50 orbital shaker (Stuart Scientific, Redhill, UK). When the OD reached 1.8-2.0, 1mL of 0.1M isopropyl-d-thiogalactopyranoside (IPTG) was added. After 5 hour induction, the cells were harvested and 2mM co factor, pyridoxal-5-phosphate (PLP) was added prior storage at -20°C following removal of broth by centrifugation. Upon thawing, the cells pellet was resuspended in 50mM HEPES buffer (pH 7.5) and 2mM PLP was added. Cells were disrupted by sonication carried out on ice using a Soniprep 150 sonicator (MSE, Sanyo, Japan) with 10 cycles of 10s, 10μm pulses with 10s intervals. The

sonicated suspension was centrifuged at 5000 rpm for 5 minutes. The resulting supernatant was then filtered through a 0.2µm filter to obtain clarified extract (cell free lysate).

Alcohol dehydrogenase from *lactobacillus kefir* (ADH): For preparation of commercial ADH, 1.2 mg/mL of ADH (0.44 U/mg) was dissolved in HEPES buffer (pH7.5) with concentrations depending on the reaction concentrations (0.5M, 1M and 1.5M) containing 10mM $MgCl_2$, as Mg^{2+} stabilizes the enzyme activity (Hummel, 1990).

Glucose dehydrogenase from *pseudomonas sp.* (GDH): For preparation of GDH, 0.02 mg/mL of commercial GDH (260 U/mg) was dissolved in HEPES buffer (pH 7.5) with concentrations depending on the reaction concentrations (0.5M, 1M and 1.5M).

2.3 ENZYME REACTIONS

CV2025 Transaminase standard reaction / Evaluation of Alternative Amino Donors: ω-transaminase bioconversions were generally carried using 0.5 mg/mL clarified lysate, 0.2mM PLP, 50mM MBA (or alternative amino donor) and 50mM Ery of 300µL total reaction volume. Reactions were carried out in 96 microwell plate and well sealed. The solutions were incubated for 20 min at 30°C prior to the addition of each amino donor and amino acceptor substrates and were then continued incubated at 30°C. The reactions were stopped by adding 0.1% (v/v) trifluoroacetic acid (TFA) prior to analysis by HPLC.

Second enzyme reaction: The CV2025 TAm/ADH/GDH coupling reactions were carried out at the same 300µL total reaction volume with 1 mM NADPH, 50mM MBA, 50 or 70mM

Ery, 70mM glucose, 0.5mg/mL CV2025 TAm, 1.5 mg/mL ADH, 0.025 mg/mL GDH, 0.2mM PLP and HEPES buffer pH 7.5 (0.5M, 1M or 1.5M) at 30°C.

Reduced pressure reaction: The 5 torr reduced pressure reactions were carried out at the same 300µL total reaction volume with 50mM MBA, 50 or 70mM Ery, 1 mg/mL CV2025 TAm, 0.2mM PLP and 50mM HEPES buffer (pH 7.5) at 30°C. For applying the 5 torr reduced pressure conditions, microwell plate was placed in an automation compatibility vacuum manifold (Sigma-Aldrich, Gillingham, UK)which was connected to the vacuum/ pressure pump (Millipore) and placed in incubator were being used.

Adsorbent resins: Reaction was carried out similar to CV2025 TAm standard reaction with the addition of 60mg of adsorbent resin and microwell was shaken at 500rpm. Prior to experiment, pretreatment procedure was conducted to wet internal pores of the resins. The dry resins were soaked with sufficient volume of methanol for 15 minutes. Then, methanol was decanted and soaked with distilled water and was again left to stand for 5-10minutes and finally replaced with distilled water and was left soaking until use.

2.4 ANALYTICAL METHODS

A Dionex (Camberly, UK) microbore HPLC system controlled by **Chromeleon** client 6.80 software was employed for all RP-HPLC analysis. This system is comprised with P680 gradient pump, ASI-100 automated sampler injector, STH 585 column oven and UVD170U UV detector.

MBA, AP and 1-phenylethanol were analyzed using an ACE 5 C18 reverse phase column (150mm x 4.6 mm, 5µm particle size; Advance Chromatography Technologies, Aberdeen, UK). A gradient was run from 15% acetonitrile/85% 0.1% (v/v) TFA to 72% acetonitrile/28% TFA over 8 min, followed by a re-equilibrium step for 2 min (oven temperature, 30°C, flow rate 1mL.min^{-1}). Detection was performed by UV absorption at 210 (MBA) and 250nm (AP). The retention times (in min) under these conditions were MBA, 3.4 min, AP, 7.28 min and 1-phenylethanol is 6.45 min.

Erythrulose (Ery) was analysed with an Aminex HPX-87H Ion exchange column (300mm x 7.8 mm, Biorad, Hemel Hampstead, UK). 0.1 % (v/v) TFA was used as mobile phase for isocratic elution with an oven temperature of 65 °C and a flow rate of 0.6 mL.min^{-1}. Detection was via electrochemical detection using a standard triple protectial, after raising the pH to 11-12 by post column addition of 0.5N NaOH (final flow rate of 1.2 mL/min). The retention time of Ery is 11.63 min.

2−amino−1,3,4−butanetriol (ABT) was analyzed using an ACE 5 C18 reverse phase column with UV detection at 254 nm and oven temperature of 37°C. Mobile phases used were A = 140 mM sodium acetate, pH 5.05 and B = 100% acetonitrile. A gradient was run from 85% A to 100% A over 10 min, flow rate 0.5 mL.min^{-1}, followed by a column wash phase and re-equilibrium step at 1 mL.min^{-1}. Samples for analysis were first derivatized using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) as the derivatizing agent. This derivatezing agent was synthesized “in house" according to the protocol detailed by Cohen and Michaud (1993). ABT samples were mixed with borate buffer and briefly vortexed. A clean tip was used to reconstituted AQC reagent, which was first prepared prior to use by mixed AQC reagent powder with dried acetonitrile as reagent diluent and heated at 55°C until

the powder dissolves. The solution was then immediately vortexed for few seconds and incubated at room temperature for 1 min prior to transfer to HPLC vial. The retention time of derivatized ABT is 8.9 min.

3. RESULTS AND DISCUSSION

3.1 STANDARD TAm BIOCONVERSION KINETICS AND YIELD

In order to illustrate the problem of low yielding equilibrium-controlled reactions initial experiments with CV2025 TAm were performed at equilmolar substrate concentrations as shown in Figure 2. Only 26% (w/w) conversions were achieved when 50mM of each substrate MBA and Ery were used in TAm bioconversion of total 300µL reaction scale.

Significant inhibition of TAm by the amine product (AP) and low equilibrium constant of the reaction prevented complete conversion as shown in Figure 3. Substrate inhibition by MBA and Ery were not observed at concentrations below 100mM (data not shown) and thus can be considered negligible. The inhibition study was performed by exogenously added the compound (i.e., MBA and Ery for substrate inhibition, AP and ABT for product inhibition) into the reaction mixture before the biotransformation reaction proceed.

To increase the product yield, four approaches which are evaluation of alternative amino donors, second enzyme system, and ISPR methods via reduced pressure reaction and adsorbent resins had been examined as depicted in Figure 1 for selectively remove AP from

reaction system, hereby eliminating its inhibition and shifting equilibrium towards the desire product, ABT.

3.2 EVALUATION OF ALTERNATIVE AMINO DONORS

Five amino donors were investigated, as the alternative for MBA compound used in the standard CV2025 TAm bioconversion for screening of amino donors comprises slighter inhibition effect and promoting irreversible reaction. As shown in Table 1, CV2025 TAm bioconversion with Ery as the amino acceptor, showed higher activities towards all tested alternative compounds with exception only for Benzylamine.

When MBA was replaced with an alternative donor compound, Isopropylamine (IPA), nearly 3-fold increase in conversion was observed representing its by-product (acetone) produced has less negative effect to the reaction. However this reaction needed a longer time, more or less double the reaction period of standard reaction using MBA. Relatively great conversions were also attained with S-*sec*-Butylamine and L-α-Serine, a naturally occurring amino acid yielded 40% and 44% (w/w) ABT respectively.

The positive results achieved shows changing amino donors are the simplest, quickest and a reliable screening method especially with micro-scale reaction for rapidly identifies many other cheap and ready sources of amine donors.

3.3 SECOND ENZYME SYSTEM

For in-situ removal of the inhibitory AP, it can be reduced to the chiral alcohol, 1-phenylethanol by applying a second enzyme, ADH. Commercially available ADH from *Lactobacillus kefir* is a NADPH-dependent enzyme and hence an effective co factor regeneration system from an economical point of view.

In principle there are two methods to recycle the cofactor, which are coupled-enzyme process and coupled-substrate approach (Faber, 2004; Peters, 1998). In the coupled-substrate process, (i.e., 2-propanol) the maximum conversion is limited by the thermodynamics of the system, as there is a competition between substrate, product, co-substrate and co-product and by inhibitions and deactivations of the newly added co-substrate to the TAm activity. Meanwhile, in the coupled-enzyme process, two independent enzymes are involved. The addition of third enzyme glucose dehydrogenase from *pseudomonas sp.* (GDH) to couple with ADH in the TAm bioconversion was chosen to recycle the cofactor NADPH as this system has high specific activity, favorable equilibrium for NADPH (Hummel, 1999), no inhibitory effect of glucose substrate to TAm activity (Yun H *et al.*, 2003) and also commercially available.

However an additional limitation of using this NADPH recycling system is that control of pH is needed because of the gluconolactone product from cofactor recycle reaction is spontaneously hydrolyzed to gluconic acid causing a pH change. Decrease of pH will severely affect the enzyme activity and consequently will decrease the whole reaction production rates. As shown in Figure 4, when asymmetric reaction was carried out with 50mM HEPES buffer (pH 7.5), the reaction conversion was saturated after 5 hour with the

production of (R)-1-Phenylethanol which attributed to the conversion of AP by ADH is 13mM, only a similar amount to the optimum conversions achieved in CV2025TAm standard reaction at 16 hour. pH measured at this particular time decreased from *CV2025* TAm optimum value of 7.5 to 4.5, a pH which TAm will certainly be deactivated. All these observations indicate that pH should be tightly control within a fairly narrow range of its optimum value.

At larger scale, a pH controller device can easily be used for controlling pH of the reaction at its optimum value (Truppo M.D *et al*., 2010) but this kind of device is not yet applicable when running a reaction using only microliter reaction volumes. Therefore an effective way of controlling pH at micro-scale reaction is need to be developed. Running reactions with strong buffer solutions, which were 0.5M, 1M, 1.5 M HEPES (pH 7.5) were investigated for achieving this purpose (Figure 5). All the substrates, co factors and enzymes apart from TAm were prepared in these targeted HEPES concentrations and were adjusted to pH 7.5 when necessary.

The reaction with the lowest HEPES concentration of 0.5M proceeded to only 50% (w/w) conversion and the pH value at the end of reaction was decreased to 7. Meanwhile, an optimum ABT production of 36mM corresponding to 72% (w/w) reaction conversion was obtained in 1M HEPES reaction with end pH value is 7.2. Finally, reaction with the highest HEPES concentration of 1.5M shown a great increase of product yield to 43mM and the pH only decreased to 7.4. Besides, the time needed for achieving this optimum yield was also quicker by 8 hours in comparison to the other two reactions of lower HEPES concentrations.

As the substrate inhibition exerted by amino acceptor (Ery) was nearly negligible (data not shown), we had examined the effect of using higher concentration deliberately for driving equilibrium towards completion of amino donor (i.e., MBA) utilization and hence a much higher desired product yield (i.e.,ABT) will be accomplished. In addition, it may also defeat the possibility if Ery is also being consumed by TAm lysate in the side reactions. When reaction in 1.5M HEPES was repeated with 70mM Ery, the 50mM MBA was about totally converted to (R)-1-Phenylethanol and 49mM ABT had been obtained at 20 hour. 40 % excess of Ery concentration had dramatically increased the initial reaction rate to nearly 2 fold of reaction rate having substrates with equal concentration molar ratio.

3.4 PHYSICAL ISPR METHODS

3.4.1 REDUCED PRESSURE

The third approach of overcoming the low equilibrium constant and ketone product inhibition is by applying reduced pressure (5 torr) conditions for encourage the evaporation of unwanted compound from the system.

This option however is only applicable when the unwanted compound (i.e., by - product) in the system is more volatile than the other substrates and target product. This is the case here in the case of the AP byproduct. The 300µL reaction scale was performed in a microwell placed in a microwell vacuum manifold as shown in Figure 1.

In micro-scale reaction, it is very important to determine the evaporation rate of reaction media (i.e., water) for accurate quantification of substrate and product concentrations. The

water evaporation profile was based on geommetric analysis. The process was carried out with the similar TAm reaction conditions as described in materials and methods section. As shown in Figure 6 the water evaporation rate was constant at 6.8μL/h during the course of the bioconversions.

The reaction profiles of the TAm reaction at 5 torr applied pressure are shown in Figure 7. As water evaporation will severely affect micro-scale reaction encompass slow reaction rate, high TAm concentration (i.e., 1 mg/mL) was used to drive reaction faster (i.e., ensuring the reaction could be completed no longer than 24 hours). When similar concentration (i.e., 50mM) of both substrates was used, the optimum ABT yield is 28mM reached at 12 hour. Again, as an alternative to enhance the reaction conversion, an excess of amino acceptor was considered. When 70mM of Ery was used, as previously observed in reaction system 1, it helped to boost the MBA conversion yielding 33mM ABT, a 40% higher than the standard TAm bioconversion reaction.

It can be deduced from the above results that the partial MBA conversion is most probably due to the inhibition effect of AP not being totally surmounted. At 5 torr pressure, the rate of AP evaporation is still considerably slow as the yields for both reactions of 50mM Ery and 70mM Ery at 2 hour are 10mM and 15mM respectively. Therefore, the pressure should be increased as well as the reaction volume in order to lessen the severe affect of water evaporation. Furthermore, increase the excess of Ery concentration is also expected to accelerate the initial rate higher, driving reaction towards completeness of MBA conversions before reaction reached its equilibrium state.

3.4.2 ADSORBENT RESINS

The fourth approach to alleviate product inhibition and low equilibrium constant problem is by in *situ* product removal using adsorbent resins for selectively binding the unwanted by-product. Four types of resins called Amberlite^{TM} XAD, which are XAD 7, XAD 1180, XAD 16, XAD 4 were investigated and the choice for application into CV2025 TAm biocoversion was based on the highest adsorption capacity for AP. Amberlite^{TM} is a hydrophobic polymer resin with wide surface area. Isopropyl alcohol was use as the organic desorbate in this study.

Based on adsorption isotherm study, the adsorption capacity for AP of all mentioned resins was much greater than MBA, Ery and ABT. From these tested resins, XAD-7 demonstrated the highest adsorption capacity for AP whilst very little adsorbing ability for both substrates and targeted product, ABT and hence was selected for application into this final process option of CV2025 TAm bioconversion reaction. Theoretically, with the concentration gradient, and taking into account the binding affinity to the substrates is not too strong, the substrates adsorbed will equilibrate to the aqueous phase and become available for reaction meanwhile AP formed diffuses into resin driving the reaction towards completion.

In order to obtain a complete reaction profile for the total amount of compounds absorbed and desorbed from the resin, the reaction was conducted in a technique called sacrifice wells. For each plotted points, sampling were done by taking off the aqueous solution from the well leaving only resin and instantaneously replaced with the same volume of Isopropyl alcohol and was left shaken for few hours to reach fully desorption capacity of the adsorbed compounds.

The CV2025 TAm bioconversion reaction with the presence of XAD 7 proceeded to only approximately 10% (w/w) conversion (data not shown), which is far lesser than the CV2025 TAm standard reaction. The unproductive result obtained from this method is mostly due to CV2025 TAm in cell free lysate form was also being absorbed by the resin and unfortunately the binding affinity was too strong to release it thus leaving a very small concentration of enzyme in the aqueous phase which was insufficient to proceed the reaction any further. Therefore, whole cell is seems to be the best option for this approach as size of the cell (i.e size of *E.*coli is 0.2-0.5 microns) is normally larger than pore size of the resin particles (approximately 0.01 microns or less) thus avoiding it from being adsorb. This approach however was not being investigated here as we are trying to show comparison for each process options with cell free lysate considering its advantages over whole cell in carry out a small scale experiment as purposely for early screening study. Cell free lysate is easy to prepare and can be stored at -20°C with no decrease in activity for months.

4. CONCLUSION

We have demonstrated the applicability to rapidly evaluate improvements in the yield of equilibrium-controlled bioconversions. The approaches were illustrated for the CV2025 TAm for asymmetric synthesis of chiral amine by four systems approaches for selectively removes an inhibitory product (AP) as well as overcoming low equilibrium constant in microscale processing technique. In comparison (Table 2), the second enzyme reaction, a system applying ADH as second enzyme and coupling with GDH for cofactor NADPH regeneration has the highest product yield than the other three investigated options. The excellent result of 86% (w/w) conversions obtained from second enzyme system with 1.5M HEPES buffer shown that CV2025 TAm is very stable in a very strong buffer solution. The evaluation of

alternative amino donors however still represents the simplest and fastest option meanwhile reduced pressure system is an efficient method for overcoming inhibition or suppresses the unfavorable equilibrium constant cause by a compound having high volatilities property. Employing second enzyme and reduced pressure systems with a higher ratio of amino acceptor to amino donor had drastically increased the reactions conversion to 98% (w/w) for second enzyme system and 66% (w/w) for reduced pressure system. For other type of applications the best process option however will depend on particular reaction being studied where there are need to be able to explore each option quickly (i.e using microwell methods).

AKNOWLEDGEMENTS

The Ministry of Higher Education of Malaysia (MOHE) and The Mexican National Council for Science and Technology (CONACYT) are acknowledged for the studentships provided to support the authors of this work. The UK Engineering and Physical Sciences Research Council (EPSRC) is thanked for the support of the multidisciplinary Biocatalysis Integrated with Chemistry and Engineering (BiCE) programme (GR/S62505/01) at University College London (London, UK). Financial support from the 13 industrial partners supporting the BiCE programme is also acknowledged.

REFERENCES

[1] Christen P, Metzler DE (1985) Transaminases. New York, *NY: Wiley.*

[2] Cohen S.A, Michaud D.P (1993) Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high performance liquid chromatography. *Anal Biochem*. **211**:279-287.

[3] Dominik K, Ivan L, Dorina C, David R, Wolfgang K (2008) Asymmetric synthesis of optically pure pharmacologically relevant amines employing ω-transaminases. *Adv Synth Catal*, **350:**2761-2766.

[4] Faber K. (2004) Biotransformations in organic chemistry. Berlin Heidelberg: Springer- Verlag.

[5] Hummel W (1999) Large-scale applications of NAD(P)-dependent oxidoreductaces: recent developments. *Trends Biotechnol*. **17**:487-42.

[6] Hummel W (1990) Reduction of acetophenone to R(+)-phenylethanol by a new alcohol dehydrogenase from *Lactobacillus kefir*. *Application Microbial Biotechnology*. **34**: 15-19.

[7] Kaulmann U, Kristy S, Mark E.B.S, Helen C. H, John M.W (2007) Substrate spectrum of ω-transaminase from *chromobacterrium violaceum* DSM30191 and its potential for biocatalysis. *Enzyme Microb. Technol.* **41**: 628-637.

[8] Lye G.J, Shamlou P.A, Baganz F, Dalby P.A, Woodley J.M (2003) Accelerated design of bioconversion processes using automated microscale technique. *Trend in Biotechnology*. **21**: 1.

[9] Peters J. (2008) Dehydrogenase Characteristics, design of reaction conditions, and applications. In: Rehm H-J, Reed G, Puhler [10] A, Stadler P, editors. Biotechnology. Weinheim: Wiley-VCH.

[10] Sheldon RA (1996) Chirotechnology designing economic chiral syntheses. *J Chem Tech Biotechnol*, **67**:1-14.

[11] Shin J.S, Kim B.G, Andreas L, Christian W (2003) Kinetic resolution of chiral amines with ω-transaminase using an enzyme-membrane reactor. *Biotechnol Bioeng*, **73**:179-187.

[12] Shin J.S, Kim B.G, Andreas L, Christian W ([2001]) Kinetic resolution of chiral amines with ω-transaminase using an enzyme-membrane reactor. *Biotechnol Bioeng*, **73**:179-187.

[13] Shin J.S, Kim B.G (1999) Assymetric synthesis of chiral amines with ω-transaminase. *Biotechnol. Bioeng.* **65**:206-211.

[14] Shin J.S, Kim B.G (1999) Modeling of the kinetic resolution of α-methylbenzylamine with ω-transaminase in a two-liquid-phase system, *Enzyme Microb. Technol*, **25**:426-432.

[15] Shin J.S, Kim B.G (1998) Kinetic modeling of ω-transamination for enzymatic kinetic resolution of α-methylbenzylamine. *Biotechnol. Bioeng*. **60:**534-540.

[16] Shin J.S, Kim B.G (1997) Kinetic resolution of α-methylbenzylamine with ω-transaminase screened from soil microorganisms: Application of biphasic system to overcome product inhibition. *Biotechnol. Bioeng.* **55**: 348-358.

[17] Shin J.S, Kim B.G (1996) Kinetic resolution of α-methylbenzylamine with α-transaminase screened from soil microorganism: Application of a biphasic system to overcome product inhibition. *Biotechnology and Bioengineering*. **55**: No. 2.

[18] Stirling DI (1992) The use of aminotransferases for the production of chiral amines and amines. In: Collins AN, Sheldrake GN, Crosby J, editors. Chirality in Industry, Uk: *John Willey & Sons*, 209-222.

[19] Sutin L, Anderson S. Bergquist L, Castro V.M, Danielsson E. James S, Henriksson, Johansson L, Kaiser C, Flyren K, Williams M (2007) *Bioorg. Med. Chem Lett*. **17**:4837-4840.

[20] Truppo M.D, Rozell J.D, Turner N.J (2010) Efficient production of enantiomerically pure chiral amines at concentrations of 50g/L using transaminases. *Organic Process Research & Development* **14**: 234-237.

[21] Yun H, Kim J, Kinnera K, Kim BG (2005) Synthesis of enatiomerically pure trans-(1R,2R)- and cis-(1S, 2R)-1-amino-2-indanol by lipase and ω-transaminase. *Biotechnol Bioeng*, **93**: 391-395.

[22] Yun H, Yang YH, Cho BK, Hwang BY, Kim BG(2003) Simultaneous synthesis of enantiomerically pure (R)-1-phenylethanol and (R)- α-methylbenzylamine from racemic α-methylbenzylamine using ω-transaminase/alcohol dehydrogenase coupling reaction. *Biotechnol Lett*, **25**: 809-814.

LIST OF TABLE LEGENDS

Table 1. The conversions of CV2025 TAm reaction with Ery and various amino donors. Each reaction mixture (300μL) contained contained 50mM Amino donor, 50mM Ery, 0.5mg/mL CV2025 TAm, 0.2mM PLP and 50mM HEPES buffer (pH7.5).

Table 2. List of productivity of the investigated process options and number of fold increases as comparison to CV2025 TAm standard reaction.

LIST OF FIGURE LEGENDS

Figure 1. Process options investigated in this work at microwell scale to overcome the yield of low equilibrium CV2025 TAm bioconversion.

Figure 2. Standard bioconversion kinetics of CV2025 TAm reaction for AP (■) and ABT (▲) productions. The reaction mixture (300μL) contained 50mM MBA (♦), 50mM Ery, 0.5mg/mL CV2025 TAm, 0.2mM PLP and 50mM HEPES buffer (pH7.5).

Figure 3. Product inhibition by AP. Reactions were carried out with 5mM MBA and 5mM Ery by addition of specified concentrations of AP (0-40mM).

Figure 4. The reaction profiles of second enzyme system for 1-phenylethanol (▲) and TAm standard reaction (the reaction mixture is as stated in Figure 3 for AP (●) production. The reaction mixture (300µL) for second enzyme system contained 50mM MBA , 50mM Ery, CV2025 TAm (0.5mg/mL), 0.2mM PLP, 1mM NADPH, 70mM glucose, ADH (1.2 mg/mL), GDH (0.02 mg/mL) and 50mM HEPES buffer (pH 7.5)

Figure 5. Second enzyme system kinetics for (A) ERY consumptions and (B) ABT production. The reaction mixture (300µL) contained 50mM MBA, Ery of 50mM in different HEPES (pH7.5) concentrations of (0.5M(■) 1M (●) or 1.5 M (▲) and 70mM Ery in 1.5M HEPES (♦), CV2025 TAm (0.5mg/mL), 0.2mM PLP, 1mM NADPH, 70mM glucose, ADH (1.2 mg/mL), and GDH (0.02 mg/mL).

Figure 6. The evaporation profile of water at 30°C under reduced pressure, 5 torr from 96 microwells. Error bars shown one standard deviation about the mean (n=6).

Figure 7. Reduced pressure bioconversion of MBA (A ----) consumptions and AP (A —), ABT (B) productions. The reaction mixture (300µL) contained 50mM MBA , Ery of 50mM (●) and 70mM (▲), CV2025 TAm (1 mg/mL), 0.2mM PLP and 50mM HEPES

LIST OF TABLES

Table 1

Amine donors	**Conv. [%]**
Isopropylamine (IPA)	72
L-α-Serine	44
S-*sec*-Butylamine	40
S-(+)-1-Aminoindane	38
S-α-Methylbenzylamine (MBA)	26
Benzylamine	12

Table 2

Option	**Final product concentration (equilmolar substrates reaction)**	**No. of fold increase**
Evaluation of alternative amino donors	36mM (IPA as the amino donor)	2.8
Second enzyme system	43mM	3.3
Reduced pressure	28mM	2.2
Adsorbent resins	5mM	-2.6

LIST OF FIGURES

Figure 1

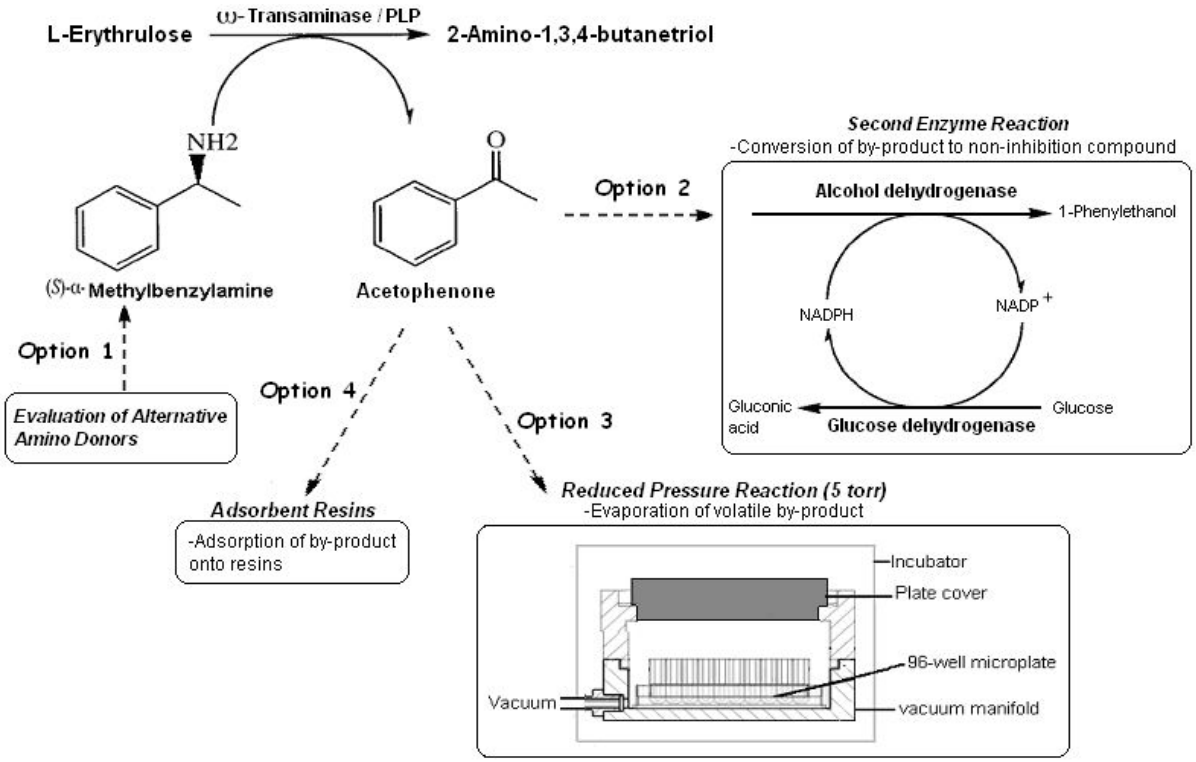


Figure 2

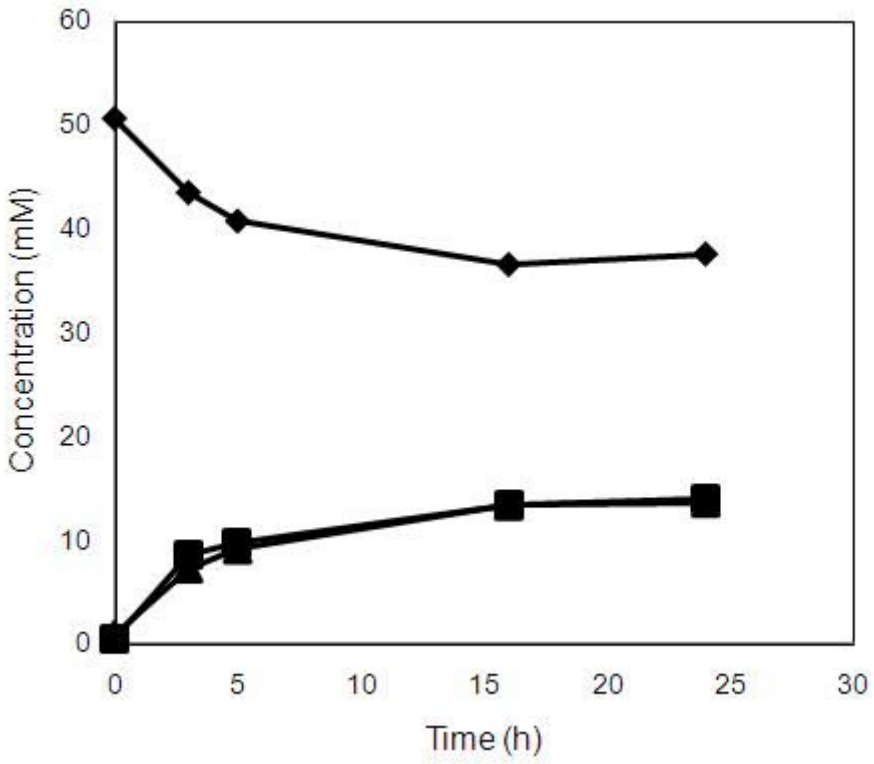


Figure 3

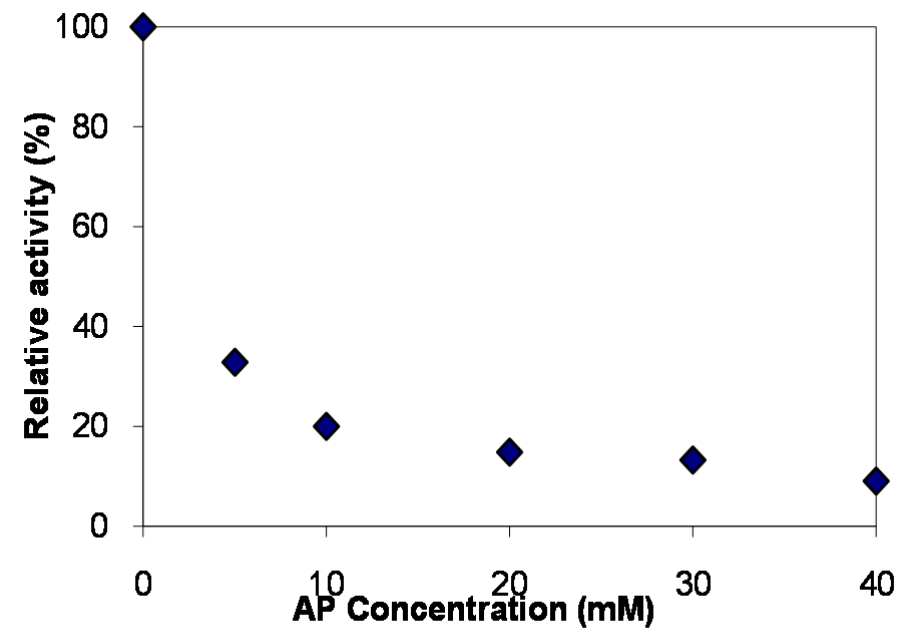

100
80
60
40
20
0
Relative activity (%)
0
10
20
30
40
AP Concentration (mM)

Figure 4

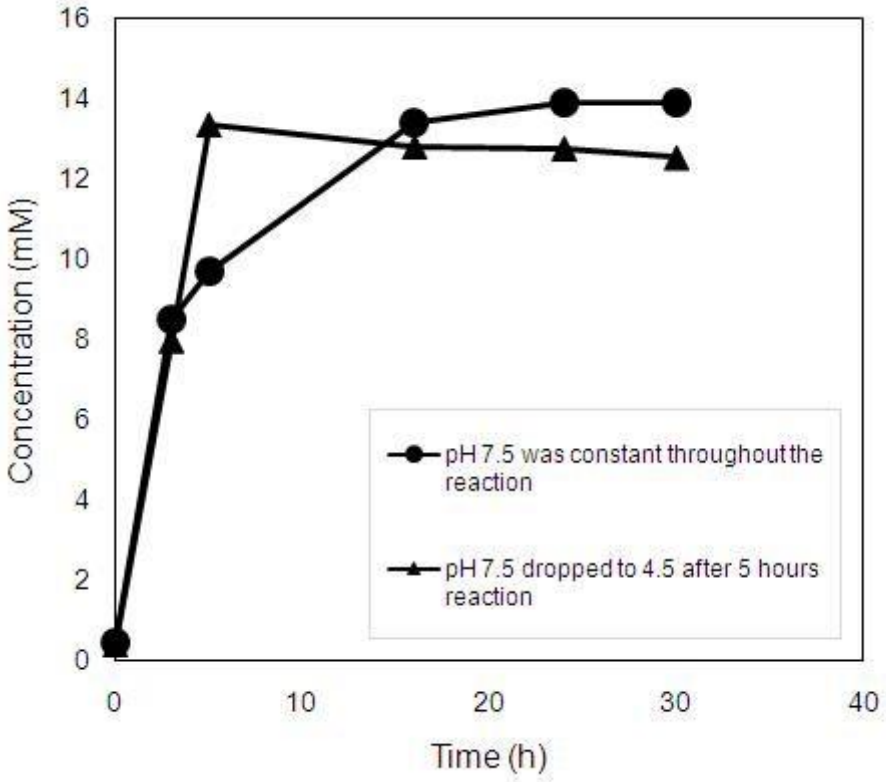

16
14
12
10
8
6
4
2
0
Concentration (mM)
0
10
20
30
40
Time (h)
pH 7.5 was constant throughout the reaction
pH 7.5 dropped to 4.5 after 5 hours reaction

Figure 5

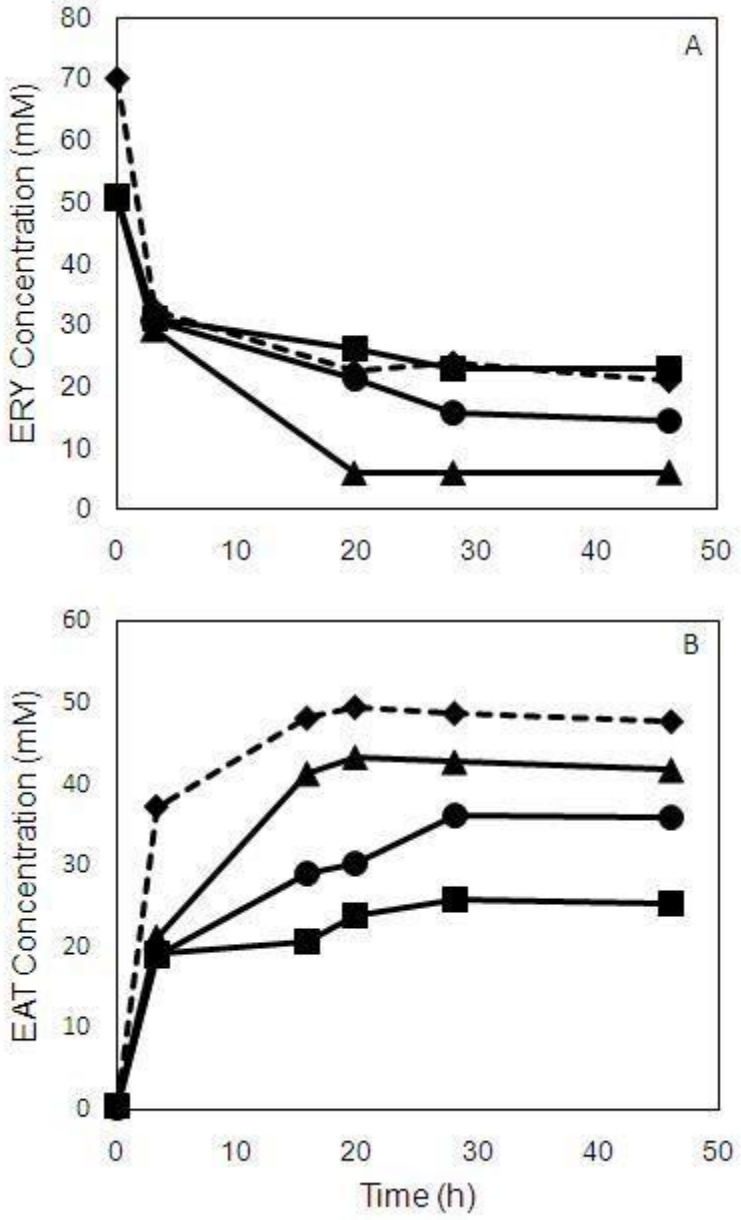

A
ERY Concentration (mM)
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
B
EAT Concentration (mM)
60
50
40
30
20
10
0
0
10
20
30
40
50
Time (h)

Figure 6

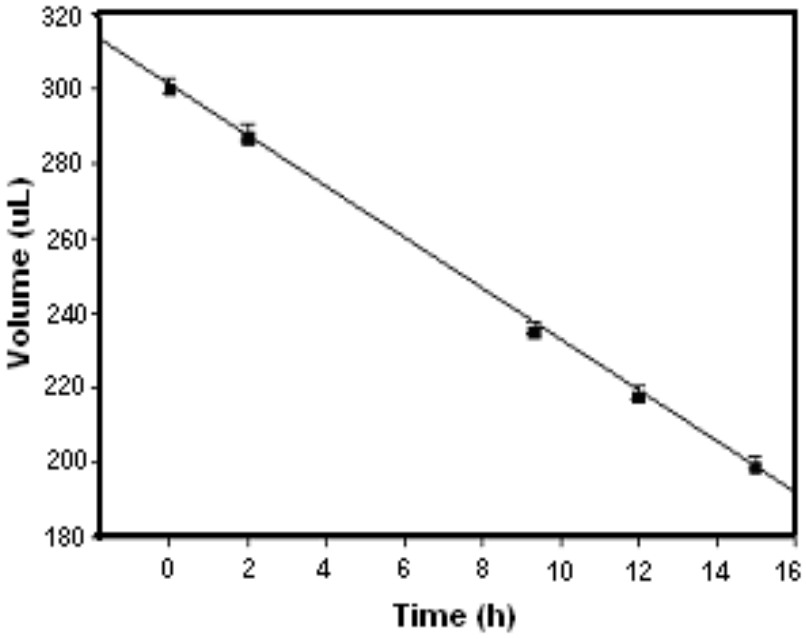
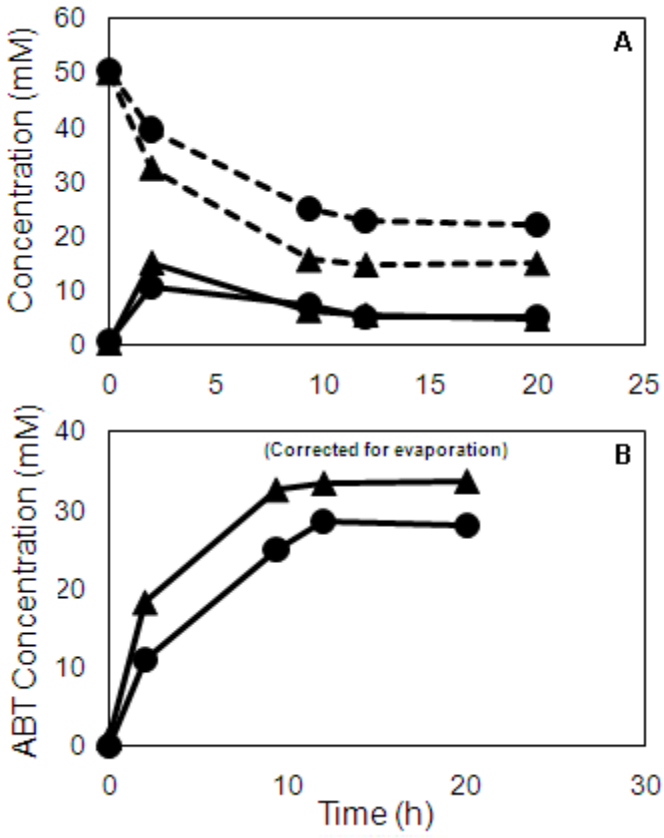

320
300
280
260
240
220
200
180
Volume (uL)
0
2
4
6
8
10
12
14
16
Time (h)

Figure 7


A
Concentration (mM)
60
50
40
30
20
10
0
0
5
10
15
20
25
(Corrected for evaporation)
B
ABT Concentration (mM)
40
30
20
10
0
0
10
20
30
Time (h)