Whole cell biotransformation for reductive amination reactions

Stephanie Klatte, Elisabeth Lorenz, and Volker F Wendisch*

Chair of Genetics of Prokaryotes; Faculty of Biology & CbBTec; Bielefeld University; Bielefeld, Germany

Keywords: whole cell biotransformation, E. coli, Corynebacterium glutamicum, Pseudomonas, alcohol dehydrogenase, transaminase, alanine dehydrogenase, cofactor recycling, glucose dehydrogenase, formate dehydrogenase

Abbreviations: G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; P5P, pentose-5-phosphate; F6P, fructose-6-phosphate; T3P, triose-3-phosphate; pgp, phosphogluconolactonase; pfKA, phosphofructokinase A; pdh, phosphofructokinase 2; sufA, glucose-6-phosphate dehydrogenase; gnd, 6-phosphogluconate dehydrogenase; dld, d-lactate dehydrogenase; ldhA, l-lactate dehydrogenase; adhE, acetaldehyde dehydrogenase; pmtA/B, subunit of pyridine nucleotide transhydrogenase; nuoG, subunit of NADH dehydrogenase I; acnC, subunit of succinyl-CoA synthetase; aceE, isocitrate lyase; favl, fumarase A; malB, malate dehydrogenase; mgs, malate-quinone oxidoreductase; MQ, menaquinone; MQH2, menaquinol

*Correspondence to: Volker F Wendisch; Email: volker.wendisch@uni-bielefeld.de Submitted 04/30/2013 Revised 11/07/2013 Accepted 11/11/2013 Published Online: 12/05/2013 http://dx.doi.org/10.4161/bioe.27151

Whole cell biotransformation systems with enzyme cascading increasingly find application in biocatalysis to complement or replace established chemical synthetic routes for production of, e.g., fine chemicals. Recently, we established an E. coli whole cell biotransformation system for reductive amination by coupling a transaminase and an amino acid dehydrogenase with glucose catabolism for cofactor recycling. Transformation of 2-keto-3-methylvalerate to l-isoleucine by E. coli was improved by genetic engineering of glucose metabolism for improved cofactor regeneration. Here, we compare this system with different strategies for cofactor regeneration such as cascading with alcohol dehydrogenases, with alternative production hosts such as Pseudomonas species or Corynebacterium glutamicum, and with improving whole cell biotransformation systems by metabolic engineering of NADPH regeneration.

Reductive Whole Cell Biotransformation: An Upcoming Tool of Biocatalysis

Biocatalysis has come more and more into focus as a green and sustainable technology for the functionalization of chemical compounds complementing and sometimes replacing established chemical synthetic routes. For the synthesis of the product of interest, chemical catalysis may be replaced by biological catalysts, which often make use of the exquisite stereoselectivity of enzymes. Biocatalysts comprise either cell-free systems with isolated enzymes or whole cells. Isolated enzymes are preferably used for hydrolytic or isomerization reactions since they are independent of costly cofactors. For reactions requiring cofactor regeneration, e.g., reduct reactions, whole cell systems are preferable since, e.g., reduct equivalents may be regenerated by cellular metabolism. Whole cell systems also offer the advantage of (1) providing the native environment, e.g., with respect to pH to keep the enzymes stable and (2) avoiding costly isolation and purification of reaction biocatalysts, intermediates, and cofactor addition. Whole cell systems using genetically amenable organisms such as E. coli, C. glutamicum, or the yeast Saccharomyces cerevisiae may be optimized for cofactor provision by genetic engineering, which will be discussed in detail later.

Whole cell biotransformations employing ATP as an energy source have been implemented less often because recycling reactions might be too complex for economical purpose. For conversion of Xanthosine monophosphate (XMP) obtained from fermentation with Corynebacterium ammoniagenes to Guanosine monophosphate (GMP), E. coli cells overproducing ATP-dependent GMP synthetase can be used and ATP can be regenerated from glucose via glycolysis by C. ammoniagenes cells.* Polyphosphate kinase, which in C. glutamicum and other bacteria also phosphorylates nucleoside diphosphates using inexpensive polyphosphate as a phospho-donor yielding NTPs was applied, e.g., for ATP recycling biotransformation of amino acids to dipeptides using 1-amino acid ligase LaL. Redox reactions have been implemented as whole cell biotransformations employing a variety of systems for redox cofactor...
reductions of ketones to alcohols, respectively, or racemization via ketone intermediates or employing electrochemical, chemical, or photochemical reactions. Here, most prominently four cofactor recycling types are highlighted due to their redox self-sufficiency of microorganisms or enzymes in the cellular metabolism.

First, a single enzyme may be used as substrate-coupled recycling system (Fig. 1A) when, e.g., reduction of a substrate such as cinnamaldehyde to the respective product cinnamyl alcohol by yeast alcohol dehydrogenase is coupled to oxidation of a second substrate such as propanol or ethanol to the respective aldehydes by the same enzyme. Second, two redox reactions generating or converting the same intermediate may be coupled with cofactor recycling (Fig. 1B). Based on this principle a general strategy for racemization of secondary alcohols under mild reaction conditions was developed by coupling a pair of stereocomplementary alcohol dehydrogenases, e.g., for racemization of (R)- and (S)-2-octanol. Redox cofactor recycling may involve coupling of different types of redox reactions, e.g., an alcohol dehydrogenase and an amino acid dehydrogenase. For conversion of α-mandelic acid to α-phenylglycine the oxidation of α-mandelic acid to phenylglyoxylylate by a t-specific alcohol dehydrogenase was coupled to the reductive amination of phenylglyoxylylate to t-phenylglycine by a t-specific amino acid dehydrogenase. As extension, addition of mandelate racemase enabled deracemization of tα-mandelic acid to tα-phenylglycine. For coupling a number of further oxidation reactions are typically used, e.g., oxidation of formate, phosphite, glucose, glucose 6-phosphate, alanine, and glutamate by the respective dehydrogenases.

Redox factor regeneration may involve coupling of a reduction reaction to cellular metabolism in a whole cell biotransformation since reducing equivalents are generated in glycolysis (Fig. 1C), e.g., coupling reduction of methyl acetocetate to (R)-methyl 3-hydroxybutyrate with cellular glucose catabolism.

Third, for the use of different cofactors (NADH, FMN, reduced ferredoxin, etc.) in the enzyme cascade, mediating enzymes are required for electron transfer (Fig. 1D). Monooxygenases that use molecular oxygen to insert one oxygen atom into a substrate while the second oxygen atom is of trimethylpyruvate to isosorbide by a leucine dehydrogenase coupled with a formate dehydrogenase for cofactor recycling (E. coli, Germany). For instance, the synthesis of L-Dopa by a tyrosine-phenol lyase in immobilized whole cell Erwinia herbicida (Ajinomoto, Japan) or the nicotinamidase production by a nitrite hydratase in immobilized Rhodococcus rhodochrous (Lonza, Switzerland) are industrially relevant biotransformations in whole cells.

For whole cell biotransformation processes the choice of the production host is important. An ideal host is genetically amenable, grows fast in simple media, allows high enzyme production levels, is recalcitrant to a wide range of substrates and products, allows efficient operation in two-phase systems, and is compatible to subsequent downstream processing regimens. For coupling of the biotransformation reactions to the host’s cellular metabolism, the ideal host allows easy import of substrates and export of products and possesses no or reduced endogenous catalytic activity toward the substrates, intermediates, and products of the biotransformation.

Due to the ease of genetic manipulation and high levels of enzyme overproduction, E. coli is the first choice as host for whole cell biotransformations. The eukaryotic baker’s yeast S. cerevisiae was screened for reductases and thus became the most common whole cell biocatalyst for reduction of β-keto-esters to chiral alcohols. Individual reductases of this microorganism are highly stereoselective and mostly NADH-specific but as the yeast produces several reductases with the same substrate specificity but different stereoselectivity, the production of enantiomeric pure products is important, e.g., pharmaceuticals became a challenging task for yeasts’ scientists.
Pseudomonas is an attractive biotransformation host due to its recalcitrance to many solvents used in biocatalytic two-phase systems like octanol and toluene, but also to toxic substrates and products, which is partly due to solvent efflux pumps such as SrpABC from Pseudomonas putida.\textsuperscript{19} Transport through the cell wall barrier is critical, e.g., for the often large and lipophilic building blocks in pharmaceutical industry such as

![Diagram of enzyme cascading for cofactor recycling in reductions and reductive aminations. Cascading substrate conversion and cofactor recycling by a single enzyme (A) or by two sequential reactions (B). Coupling of a reduction reaction with glucose-driven cofactor regeneration by the cellular metabolism directly (C) or via an electron transfer mediator such as ferredoxin (D). Coupling a transaminase reaction and an amino acid dehydrogenase to either an alcohol dehydrogenase (E) or cellular glucose catabolism (F).]
Figure 2. Metabolic engineering of host cell metabolism for improved biotransformations. NADPH regeneration can be improved by forcing glucose catabolism to the oxidative pentose phosphate pathway (PPP) by deletion of pgI, the gene for phosphoglucoisomerase (A) or by deletion of the genes for phosphofructokinase genes pfkA and pfkB resulting in partial cyclization of the PPP (B). Improved biotransformation capacity resulted from various gene deletions primarily encoding tricarboxylic acid enzymes (C).
hydroxylated long chain fatty acids.

Heterologous production of, e.g., the fatty acid uptake system of *Pseudomonas oleovorans* in *E. coli*, improved hydroxylation of pentadecanoic acid. Alternatively, cell permeabilization, e.g., by the bacteriostatic anticybacterial drug ethambutol, improved cyclohexanone whole cell biotransformation by *C. glutamicum*. This chemically modified bacterium is attractive as whole cell catalyst as its mycolic acid harboring cell wall protects against toxic compounds. It has a history of five decades of safe use in the food and feed industries for the annual production of 2,930,000 tons of L-lysine (Ajinomoto, Inc., available at: http://www.ajinomoto.com/en/ir/pdf/FY13Q1_data_E.pdf, cited 05 September 2013).

Metabolic Engineering of Biotransformation Hosts

The reductive whole-cell-based biotransformation processes for production of steroselective chemical and pharmaceutical compounds, amino acids, chiral alcohols, and fine chemicals, have developed rapidly through process optimization and engineering of cellular metabolic pathways. Metabolic engineering focused on core biosynthetic pathways, cofactor-regeneration systems, uptake and export systems, and further optimization of the cellular interaction. For whole cell biotransformation involving redox reactions, regeneration of reduction equivalents, mostly the nicotinamide adenine dinucleotide coenzymes NADH and NADPH, is critical.

With *E. coli*, different strategies for cofactor regeneration have been applied, e.g., cascading of reductive biotransformations with oxidation of formate to carbon dioxide by NADP(H)-dependent formate dehydrogenase. Glucose dehydrogenase (GDH) from *Bacillus subtilis* enhanced NADP(H)-dependent biotransduction of ethyl 4-chloro-3-oxobutanate (COBE) to ethyl (R)-4-chloro-3-hydroxybutyranate (CHBE). Coupling to cellulosic metabolism for cofactor regeneration can be optimized by metabolic engineering, e.g., for NADPH generation. Since two molecules of NADPH are generated in the oxidative pentose phosphate pathway (PPP), overproduction of glucose-6-phosphate dehydrogenase (G6PDH) in engineered *E. coli* improved 6-epi-cápolonacetic production. Alternatively, the competing pathway glycolysis may be abrogated by deletion of the gene of the first glycolytic enzyme phosphoglucone isomerase (pgi) such that each imported glucose molecule is catabolized via the oxidative PPP before entering glycolysis at the levels of fructose 6-phosphate and triosephosphates (Fig. 2A).

The subsequent increase of NADP(H) regeneration improved production of two polyphenols, leuconosidin and catechin, from dihydroferulic acid. Extending this concept, the oxidative PPP was partially cycled by deletion of the genes for phosphofructokinases *pfkA* and *pfkB*, which entails that only glyceraldehyde-3-phosphate produced in the PPP enters glycolysis while fructose 6-phosphate re-enters the oxidative PPP (Fig. 2B). This partial cycling enhanced the reduction of the prochiral ketone methyl acetoacetate (MAA) to the chiral hydroxy ester (R)-methyl-3-hydroxybutyrate (MBH).

In order to investigate the metabolic redox capacity of recombinant *E. coli* for asymmetric styrnne epoxides deletion, mutants lacking genes relevant for the NADH regeneration were compared in silico and in vivo according to NADH regeneration rates. Based on this approach, several genes that are directly involved in NADH regeneration were selected to assess their potential to increase reductive amination of keto acids. For example, we have described an *E. coli* whole cell biotransformation system for reductive amination of 2-keto-3-methylvalerate to 3-isoleucine (Fig. 2C).

Amino Acid Production

Amino acids are commercially important as they are widely used as additives in food, feed, pharmaceuticals, cosmetics, polymer, and other industries with an annual market growth rate of 5–7% worldwide. Natural proteinogenic amino acids are mostly produced from sugars by fermentation. For the production of non-natural amino acids and their derivatives, whole cell biotransformations can be employed. Non-natural amino acids find application as synthons for chemical synthesis of active pharmaceutical ingredients (APIs) and as orthogonal modules for genetic code expansion in synthetic biology. Enzyme-catalyzed amino acid production may proceed via ammonia addition to allenynes by ammoxanases.

Commercial production of L-tert-leucine is based on aspartate ammonia lyase enzyme, which catalyzes the double bond of fumaric acid to yield L-tert-leucine and, therefore, does not depend on reduction equivalents such as NADPH. The aspartate ammonia lyase process is also the basis for commercial L-tert-leucine production since the L-tert-leucine obtained from fumarate can be decarboxylated to L-tert-leucine. Transamination or reductive amination of keto acids may be pursued by enzyme catalysis or by whole cell biotransformation. Amino acid dehydrogenases catalyze reductive amination using ammonia in an NAD(P)H-dependent manner. These enzymes are rather substrate specific, such as L-2-oxalylglycine dehydrogenase, L-glutamate dehydrogenase or L-phenylalanine dehydrogenase, which are active with a broad spectrum of keto acids as substrates, but they require stoichiometric supply of an amino acid as amino group donor. Amino acid dehydrogenases may be coupled with transaminases to enable reductive amination of many keto acids. For example, we have described an *E. coli* whole cell biotransformation system for reductive amination of 2-keto-3-methylvalerate (KMV) to the amino acid L-isoleucine by coupling endogenous L-alanine-dependent transaminase AsA/ND with NADH-dependent L-alanine dehydrogenase from *B. subtilis*. Recycling of the cofactor NADH was ensured by glucose catabolism of the host *E. coli*. Thus, the *E. coli* host which produces heterologous NADH-dependent L-alanine dehydrogenase and catabolizes glucose.
may serve as a chassis for a wide range of reductive amination reactions by coupling to the respective transaminase.

This concept of reductive amination, however, is dependent on the provision of the keto acids, e.g., by chemical synthesis, as precursors of the desired amino acids. Both the non-natural keto acid or the non-natural amino acid may be toxic to the reductive amination host cell as observed for the keto acids ketaisocaproate and ketaisovalerate, therefore, use of alternative hosts such as C. glutamicum or Pseudomonas may be needed. Moreover, uptake of the non-natural keto acid into the host cell and export of the produced non-natural amino acid out of the cell may slow or even preclude effective reductive amination and may necessitate using alternative hosts or co-expression of transport genes. Currently, redox factor recycling only occurs by glucose catabolism. Besides coupling to favorable oxidation reactions such as formate dehydrogenase which drive the reaction to completion since the generated carbon dioxide gases out, the reaction may start with a hydrosyde acid instead of the keto acid. Using enzyme preparations, a transaminase/l-alanine dehydrogenase pair has been coupled to an alcohol dehydrogenase, which recycles the reduction equivalent and provides the precursor for the transaminase. This three-enzyme-cascade catalyzes redox-neutral amination of an alcohol to the amine. It should be possible to transfer this principle to a whole cell biotransformation setup for amination of hydroxyl acids to amino acids.

**Outlook**

Our demonstration of efficient reductive transamination in an E. coli whole cell biotransformation system with the enzyme couple alanine dehydrogenase AlaDH and transaminase TAT AvrA provides a basis for further applications. While shown exemplarily for amino acid production, it should be feasible to transfer this approach more broadly to production of amino functionalized chemicals. Redox cofactor regeneration via glucose catabolism by the biotransformation host E. coli was improved by generally engineering its metabolism. As discussed here, alternative redox cofactor recycling systems—e.g., by coupling to oxidation reactions catalyzed by glutamate dehydrogenase and formate dehydrogenase—may be employed to enhance the described whole cell biotransformation system. Moreover, reductive amination by whole cell biotransformation may involve alternative hosts, such as the most solvent resistant pseudomonads or C. glutamicum, which has been used safely in food and feed industries for more than 50 years, since these alternative hosts are also amenable to genetic and metabolic engineering.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

1. Schwalb A, Dotzek JS, Haer A, Kienz A, Walbohms M, Walbohms B. Industrial biocatalysis today and tomorrow. Nature 2001; 409:236-8. PMID:11199457; http://dx.doi.org/10.1038/35097579

2. Bajo T, Nishi T, Fur S, Marnapina A. High level expression of XMP amine oxidase in Echerichia coli and its application for the industrial production of γ-aminobutyric acid. Biores Biorecharc 1997; 61:840-5. PMID: 9787961; http://dx.doi.org/10.1271/bcbp.1997.61.840

3. Lindner SN, Vidame D, Walbohms S, ,, Walbohms R. Novel, NAD-PH-dependent α-keto-β-methylaspartate reductase. J Bacteriol 2006; 188:2592-9. PMID: 16845256; http://dx.doi.org/10.1128/ JB.06404-05

4. Wendisch VF. Amino Acid Biotransformations: Pathways, Regulation and Metabolic Engineering. Springer, 2007.

5. Schrittwieser JR, Sato J, Barch V, Mork PG, Kronert W. Recent advances in shaletransformation cascade. Crit Opin Chem Biol 2011; 15:269-56. PMID:21389324; http://dx.doi.org/10.1016/j.cob.2011.03.001

6. Gopin H, Ganeshkumar P, Onogidh N, Railakhan C, Draus K, Hummel W, Walbohms A, Oy M. Exoenzymatic reduction of ketones with “designer cells” at high substrate concentrations: highly efficient access to functionalized optically active secondary alcohols. Angew Chem Int Ed 2006; 45:567-81. PMID:16878766; http://dx.doi.org/10.1002/anie.200503939

7. Walbohms R, Yose-Radchi D. Cofactor regeneration at the rib level. Adv Biochem Eng Biotechnol 2005; 89:59-118. PMID:15770539; http://dx.doi.org/10.1007/1-4020-89911

8. Zucco P, Liberati M, Bossini A, Sanzian E. Cofactor recycling for selective enzymatic racemisation of unsymmetrical to symmetrical alcohol. Biores Biorecharc 2005; 73:1224-5. PMID:16268068; http://dx.doi.org/10.1271/bcbp.16268068

9. Graber CS, Noel BH, Gross J, Hildebrandt F, Berners-Price UT, Faber K, Kronert W. Erosion of racemate activity by coupling to a pair of enantiocomplementary biocatalysts. Chemistry 2007; 13:627-14. PMID:17559936; http://dx.doi.org/10.1002/chem.200705028

10. Sculpic K, Ruchel B, Hummel W, Kiel MR, Vit T, Ruge AM, Wandsch C, Krugl UC. A novel, efficient, new method of NADPH recycling in an enzyme biotransformation of cinnamaldehyde to cinnamyl alcohol. Biosci Biotechnol Biochem 2009; 73:1224-5. PMID:19383825; http://dx.doi.org/10.1271/bcbp.2009005100

11. Sato J, Tsuda M, Tabata K, Matsu FH, Faber K, Pflüger J, Hase T, Kronert W. Redox self-sufficient biocatalytic network for the amination of primary alcohol. Angew Chem Int Ed 2012; 51:8356-60. PMID:22885745; http://dx.doi.org/10.1002/anie.201204483

12. Sudol S, Biringer S, Bert M. Increased NADH availability in Echerichia coli: improvement of the product per glucose ratio in reductive whole-cell biotransformation. Appl Microbiol Biotechnol 2011; 92:929-37. PMID:21670981; http://dx.doi.org/10.1002/biot.21837

13. Bühler B, Stuber S, Bringer S, Bott M. Increased NADPH availability limits asymmetric biocatalytic epoxidation in a growing recombinant Escherichia coli strain. Appl Environ Microbiol 2008; 74:1436-40. PMID:18192422; http://dx.doi.org/10.1128/AEM.00600-07

14. Biocatalyst engineering by assembly of fatty acid biotransformatios and stereoselective Biohydroxylations with a P450(pyr) Monooxygenase of Sphingomonas ramosa. Appl Environ Microbiol 2008; 74:3784-9. PMID: 18278480

15. Kiefer S, Dräger J, Zellner O, Wuttke T, Wettken U. Advances in biocatalytic oxidation-reduction biocatalysis today and tomorrow. Nature 2001; 411:840-5. PMID:9178561; http://dx.doi.org/10.1038/35097557

16. Wanih B, Walbohms R. Reductive amiation by recombinant Echerichia coli. Whole cell biotransformation of 3-keto-2-methylbutyrate to the corresponding amino acid. J Biotransform 2008; 16:289-94. PMID:18341575; http://dx.doi.org/10.1080/10603760802136164

17. Johannes T. Biotransformation.London, UK: Taylor & Francis, 2006. Encyclopedia of Chemical Processing.

18. Stewart JD. Organic transformations catalyzed by engineered yeast cells and related systems. Curr Opin Biotechnol 2008; 15:361-8. PMID:17877842; http://dx.doi.org/10.1016/j.cob.2008.04.011

19. Klenkho J, Dräger J, Zellner O, Meier A. Active effects of organic solvents by pseudomonas epiolide. J Biotechnol 1996; 48:678-72. PMID:9582529

20. Schmid B, Walbohms SK, Szargel D, Walbohms B. Biocatalyst engineering by assembly of fatty acid transport andoxidation activities for in vivo application of cyanobacteria P-480-type mesoporous. Appl Environ Microbiol 1998; 64:3784-9. PMID:9776000

21. Yao J, Lee J, Yang X, Che S, Kim A, Kim Y, Park JH. Ethambutol-mediated cell wall modification in recombinant Cyanobacterium glaucescens increases the biotransformation rate of cis,cis-syn-4,4-decadienal. Bioresource Technol 2012; 115:21-4. PMID:22006677; http://dx.doi.org/10.1016/j.biortech.2012.03.010

22. Tanne S, Walbohms M. Advances in biocatalytic synthesis of pharmaceutical intermediates.Curr Opin Chem Biol 2010; 15:188-94. PMID:20010403; http://dx.doi.org/10.1016/j.cob.2010.02.007
23. Ishiy T, Honda K, Shimizu S. Whole organism biocatalysis. Curr Opin Chem Biol 2005; 9:174-80; PMID:15811802; http://dx.doi.org/10.1016/j.cbi.2005.02.001

24. Duan W, van Beilen JB, Witholt B. Using proteins in their natural environment: potential and limitations of microbial whole-cell fermentation in applied biocatalysis. Curr Opin Biotechnol 2003; 14:419-25; PMID:12954157; http://dx.doi.org/10.1016/S0958-1669(03)00123-4

25. Xu Z, Jiang R, Liu Y, Cao P. High-level expression of recombinant glucose dehydrogenase and its application in NADPH regeneration. J Mol Microbiol Biotechnol 2007; 9:61-9; PMID:17411638; http://dx.doi.org/10.1159/000101182

26. Lee WH, Park JB, Park K, Kim MD, Seo JH. Enhanced production of ε-caprolactone by overexpression of NADPH-regenerating glucose-6-phosphate dehydrogenase in recombinant Escherichia coli harboring cyclohexanone monooxygenase gene. Appl Microbiol Biotechnol 2007; 76:629-38; PMID:17547576; http://dx.doi.org/10.1007/s00253-007-1016-7

27. Chemler JA, Fowler ZL, McHugh KD, Keffer MG. Improving NADPH availability for natural product biosynthesis in Escherichia coli by metabolic engineering. Metab Eng 2011; 12:96-104; PMID:21464846; http://dx.doi.org/10.1016/j.ymben.2010.07.003

28. Siedler S, Bringer S, Best M. Increased NADPH availability in Escherichia coli: improvement of the product per glucose ratio in reductive whole-cell biotransformation. Appl Microbiol Biotechnol 2011; 90:525-37; PMID:21431991; http://dx.doi.org/10.1007/s00253-010-3374-4

29. Lindeberg M, Hellmacher K, Deitz K. Biotenechnological production of amino acids and derivatives: current status and prospects. Appl Microbiol Biotechnol 2005; 69:1-8; PMID:16074792; http://dx.doi.org/10.1007/s00253-005-0155-y

30. Verstuiven J, Meulens H, Mentink S, Alberschot JAC. Second-Generation process for the synthesis of L-ornithine/L-lysine: Asymmetric reductive amination using a recombinant whole cell catalyst. Org Process Res Dev 2004; 8:461-6; http://dx.doi.org/10.1021/op0351702