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Research review paper

Rules for biocatalyst and reaction engineering to implement effective, NAD(P)H-dependent, whole cell bioreductions

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ABSTRACT

Access to chiral alcohols of high optical purity is today frequently provided by the enzymatic reduction of precursor ketones. However, bioreductions are complicated by the need for reducing equivalents in the form of NAD(P)H. The high price and molecular weight of NAD(P)H necessitate *in situ* recycling of catalytic quantities, which is mostly accomplished by enzymatic oxidation of a cheap co-substrate. The coupled oxidoreduction can be either performed by free enzymes in solution or by whole cells. Reductase selection, the decision between cell-free and whole cell reduction system, coenzyme recycling mode and reaction conditions represent design options that strongly affect bioreduction efficiency. In this paper, each option was critically scrutinized and decision rules formulated based on well-described literature examples. The development chain was visualized as a decision-tree that can be used to identify the most promising route towards the production of a specific chiral alcohol. General methods, applications and bottlenecks in the set-up are presented and key experiments required to "test" for decision-making attributes are defined. The reduction of *o*-chloroacetophenone to (*S*)-1-(2-chlorophenyl)ethanol was used as one example to demonstrate all the development steps. Detailed analysis of reported large scale bioreductions identified product isolation as a major bottleneck in process design.

Contents

1. Introduction . . . 1642
   1.1. Scope of the review . . . 1643
2. Development chain of bioreductions . . . 1643
   2.1. Enzyme sources . . . 1643
      2.1.1. Screening of microbial hosts . . . 1643
   2.2. Catalyst level . . . 1643
      2.2.1. Native hosts . . . 1644
      2.2.2. Recombinant *E. coli* . . . 1644
      2.2.3. Limitations of whole cell catalysts . . . 1644
   2.3. Reaction level . . . 1645
      2.3.1.1. In situ *substrate supply (isss) and product removal (ispr)* . . . 1645
   2.4. Diagnostic parameters . . . 1645
   2.5. Practical use of the decision tree . . . 1646
3. Methods and applications . . . 1646
   3.1. Screening of microbial strains . . . 1646
   3.2. Enzymes from databases . . . 1647
   3.3. Analytics . . . 1647
   3.4. Reductase activities . . . 1647
   3.5. Design of whole cell catalysts based on *E. coli* . . . 1647
      3.5.1. Duet vectors . . . 1648
      3.5.2. Two plasmids . . . 1648
      3.5.3. Scale-up of biomass production from shaken flask to bioreactor . . . 1648

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3.6. Reaction . . . 1649
3.6.1. Comparison of strains . . . 1649
3.6.2. Reaction optimization . . . 1650
3.7. Scale-up of whole cell bioreductions . . . 1650
3.7.1. Product isolation . . . 1651
Funding . . . 1651
References . . . 1651

1. Introduction

Numbers one, two, three and nine out of the 10 top-selling drugs in history are non-peptidic, enantiopure molecules (data from October 2013; Nixon, 2013) and chiral compounds will, as reckoned by analysts, still have a prominent position on blockbuster drug lists by 2020 (Brown, 2014). Single-enantiomer pharmaceuticals are typically administered in optical purities of 98% e.e. and above (Pollard and Woodley, 2007). Such enantiomeric purities are best obtained from enzyme-catalyzed reactions. Hence, there is a strong drive to implement biocatalytic steps into synthetic routes towards many pharmaceutical products (Wohlgemuth, 2007). Enantiopurity is generally obtained either by synthesizing specifically one enantiomer or resolving a racemic mixture. The quest for synthetic efficiency naturally favors

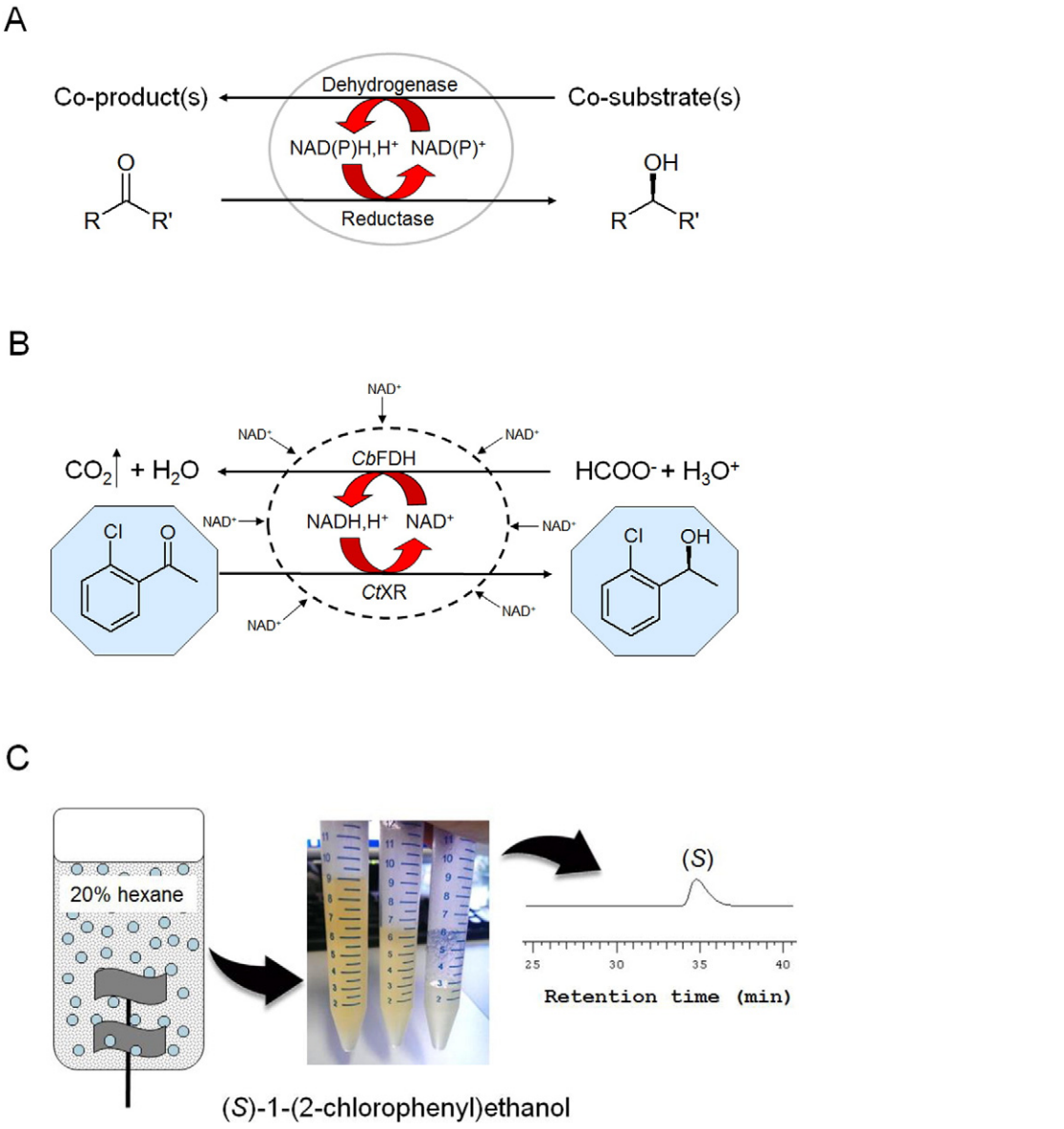

Fig. 1. General scheme of bioreductions catalyzed by free enzymes or whole cells (gray oval indicates the cell envelope) (A). Whole cell reduction of *o*-chloroacetophenone catalyzed by recombinant *E. coli* based on *Ct*XR and *Cb*FDH (the dashed oval line depicts cell permeabilization, the blue hexagons illustrate *i*sss and *i*spr by a water immiscible co-solvent). (B). Scheme of the multiphasic *o*-chloroacetophenone bioreduction at 0.5-L scale. The reaction was performed in a stirred tank reactor with pH and temperature control (gray points depict the biomass, blue drops show the hexane phase extracting *o*-chloroacetophenone and (*S*)-1-(2-chlorophenyl)ethanol). The three tubes show the extracted (*S*)-1-(2-chlorophenyl)ethanol that was obtained per batch (20 g) and that was further analyzed by chiral HPLC (C).