Enantioselective Microbial Reduction with Baker's Yeast on an Industrial Scale

Martin Bertau* and Martin Bürl 

Abstract: Microbial synthesis is an important contribution to Green Chemistry and production-integrated environmental protection. The example of baker's yeast is used to demonstrate how microorganisms can be versatile reagents for asymmetric synthesis and how microbial technologies can be alternatives and complements to catalytic processes. The commercial viability of enantioselective microbial processes on an industrial scale is shown with the examples of (S)-3-hydroxybutyric acid ethylester (1), (2S,5S)-hexanediol (2) and (1R,2S)-cis-2-hydroxycyclohexane carboxylic acid ethylester (3). The investigation of several competing enzymatic pathways in the living cells during the reduction reaction allows the process to be controlled and makes this technology applicable for the large-scale commercial synthesis of 3.

Keywords: Baker's yeast· Chiral pharmaceutical intermediates· Commercial chiral biosynthesis· Green chemistry· Microbial reduction

Microbes in Industrial Chemistry

Manifold applications for microbes in industrial chemistry exist. They serve as suppliers of products of value, active ingredients and enzymes. Of steadily growing importance are two additional applications: microbes as synthetic tools and microbes in exhaust air treatment. Bacteria, fungi or plant cells are utilised in biotechnology applications. Most processes work under sterile conditions; indispensable in the case of genetically engineered microorganisms. The search for applicable solutions for microbial conversions on a commercial scale led us to the use of baker's yeast (Saccharomyces cerevisiae) which is readily available, cheap, and, what has to be stressed, can be used under non-sterile conditions.

Baker's yeast is common in a series of industrial applications, the most familiar ones being brewing and wine-making or bakery. Yeast is also involved in bioethanol production [1].

At Rohner, baker’s yeast serves as a reagent for organic-chemical syntheses, namely the production of chiral pharmaceutical intermediates for the life-science industry [2]. Thus, yeast technology allows elegant and easy access to a number of important key intermediates for enantioselective synthesis.

Bioreduction and Catalytic Hydrogenation

The synthetic potential of microbiological methods is commonly judged in comparison with asymmetric catalytic hydrogenation technology. Clearly, the limitation of biotechnological methods to often only one stereoisomer and the lower productivity is disadvantageous. Many conventional syntheses fail due to incompatibilities with the catalyst material. The harsh conditions often applied in conventional asymmetric synthesis are circumvented smoothly, allowing even sensitive compounds to be submitted to enantioselective transformations.

Stereoselective biohydrogenation of prochiral keto compounds is not the only type of reduction reaction for which baker's yeast is well suited. Moreover it is a multifunctional reagent, applicable for further stereoselective C=C reductions [3] and several C–C bond forming reactions [4]. The ensemble of catalytic hydrogenation and microbial methods covers a broader technological range than the single technologies themselves.

From this it is evident that microbial and catalytic asymmetric hydrogenation technologies complement each other in an ideal manner.

Yeast Technology: Green Chemistry

The use of baker's yeast in industrial chemistry is a form of production-integrated environmental protection [5] and it is a textbook example of how Green Chemistry is viable on a commercial scale. Only tap water, commercial baker's yeast and a carbon-source such as cane sugar or glucose are needed. Thus the fermentation process involves solely the regeneration of raw material. Efficient recovery systems allow a considerably lower loss of volatile organic compounds (VOC), rendering the downstream processing ecologically and economically attractive [6]. The ecological advantages of microbial syntheses with baker's yeast can be summarised as follows:
Yeast and sugars regenerate raw material
Water is an environmentally friendly solvent
No or little organic loading (VOC) of exhaust air
No or little wastewater pollution load
Disposal of solid waste (biomass) is unproblematic
Organic solvents are recovered to a high degree
Chemistry free from heavy metals
Therefore, yeast chemistry is Green Chemistry.

All this demonstrates that microbial chemical conversions are also attractive from an economical point of view. The negligible pollution of air and water, high recovery degrees of VOC and the employment of readily available and cheap raw materials render this highly ecological technology inherently economical. Furthermore, instead of thermal treatment, organic exhaust air loads are degraded biotechnologically with a biofilter (Biovent®) [7].

Examples of Commercial Chiral Biosyntheses

The effective and powerful potential of microbial syntheses will be demonstrated with three examples of chiral building blocks:

1. The large-scale synthesis of (S)-3-hydroxybutyric acid ethylester (1) shows how a microbial process can be more cost-effective than a catalytic variant.

2. The case of (2S,5S)-hexanediol (2) is an example of how a microbial route is technologically superior to other variants and simultaneously the most economical method.

3. Finally, (1R,2S)-cis-2-hydroxycyclohexane carboxylic acid ethylester (3) is an example of a reduction that is only viable on a commercial scale by means of biotechnology.

Example 1: Bioreduction as a Cost-effective Variant

Hydroxybutyric acid ethylester (1) is a highly versatile pharmaceutical intermediate for chiral drug synthesis (Scheme 1).

The production of 1 by means of biotechnology is economically more favourable than production via catalytic hydrogenation. Due to its better space-time yield, the productivity of the catalytic variant increases on a very large scale (e.g. >1mt), as the costs for infrastructure and the catalyst material are compensated. Table 1 compares both alternatives.

Table 1: Comparison of the biotechnological production of 1 vs. the catalytic alternative

<table>
<thead>
<tr>
<th>Baker's Yeast</th>
<th>Catalytic Hydrogenation [8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (500 ml)</td>
<td>Methanol (40 ml)</td>
</tr>
<tr>
<td>Baker's Yeast (50 g)</td>
<td>Ru-BINAP (0.4 g)</td>
</tr>
<tr>
<td>Sugar (100 g)</td>
<td>H₂ (20–100 bar)</td>
</tr>
<tr>
<td>Ambient temperature (20–50 h)</td>
<td>Ambient temperature (40 h)</td>
</tr>
<tr>
<td>Three-step work-up</td>
<td>Two-step work-up</td>
</tr>
<tr>
<td>60–75% Yield</td>
<td>98% yield</td>
</tr>
<tr>
<td>(S)-Enantiomer, ee &gt;98%</td>
<td>(R)- or (S)-Enantiomer, ee &gt;99%</td>
</tr>
<tr>
<td>Space-time yield: -1.2 gi⁻¹ h⁻¹</td>
<td>Space-time yield: -12.2 gi⁻¹ h⁻¹</td>
</tr>
</tbody>
</table>

In the case of 1, the limitation of the fermentation route to one stereoisomer is not disadvantageous, as the (R)-enantiomer ent-1 is readily accessible by alcoholyis of (R)-polyhydroxybutanoate (PHB) [9]. The latter is also produced by microbial means – in this case as a bacterial energy store. Bacteria, such as Alcaligenes eutrophus, accumulate (R)-PHB to more than 90% of their dry cell-weight [10]. The synthesis of ent-1 from (R)-PHB is less expensive than by fermentation pathways.

This is a clear example of how the limitation of only one stereoisomer can be overcome by the synergistic combination of biotechnological methods.

Example 2: More Competitive and More Efficacious than Alternative Processes

(2S,5S)-Hexanediol (2) serves as precursor of chiral five-membered ring heterocycles as ligands for asymmetric hydrogenation catalysts, such as DuPHOS [11][12]. For the production of 2 several procedures are described. The best reported catalytic route uses (S)-BINAP-Ru at 67 bar and affords a 4:1 mixture of the cis-product 2 and its meso-stereoisomer in only moderate yields directly from diketone 4 [13]. Other variants suffer from the high synthetic effort necessary and furnish 2 in unsatisfactory yields.
and stereoselectivities [14]. All catalytic procedures have the common disadvantage that they are not commercially viable. Enzymatic methods involve racemic NaBH₄-reduction of the diketone precursor 4. The lipase-mediated stereoselective acylation of the (R)-configured alcohols furnishes the (S,S)-isomer in usually high ee, but in some cases in rather low diastereoselectivity [15]. Low conversion rates and long reaction times make enzymatic routes unfavourable.

A better alternative is a three-step procedure which circumvents the low stereoselectivity of the Ru-BINAP system for acetonilacetone (4) by using methyl acetacetate (5) as a substrate. Saponification to 3-hydroxybutyric acid (6) is followed by a Kolbe-electrolytic decarboxylative dimerization to the respective hexanediol [16]. This process is suitable for the production of (S,S)-2 and the (R,R)-enantiomer ent-2 respectively. Although the enantiopurity of the product is high (ee >99%), this method suffers from the rather poor yield of the last step (55%).

The baker's yeast reduction of 4 furnishes 2 directly in a one-step process in up to 90% yield (Scheme 2). With an ee and de of 99.9% each, this process is currently the most powerful method to access enantiopure 2 in high yield and high purity (>99.5%). With only half the time effort and twice the yield of the catalytic alternative, the biotechnological variant produces 2 with almost 100% selectivity. These results demonstrate how a biotechnological process can be ecologically attractive and economically superior to classical routes.

**Selectivity**

The formation of 2 is a highly selective process. Side-reactions are observed to only a marginal extent. Exact steering of the microbial process allows the circumvention of the competing ring-closure reaction of intermediate 7 to the five-membered ring species 8. This side-path bears complications as the elimination of water to dimethyldihydrofurane 9 is irreversible (Scheme 3).

**Example 3: Commercially Accessible by Biotechnological Means Only**

(1R,2S)-cis-2-Hydroxycyclohexane carboxylic acid ethylester (3) has manifold applications, such as a precursor in natural product synthesis [17], for chymotrypsin inhibitors [18] and for carbapenems [19]. There are currently three technologies for the synthesis of 3. The asymmetric hydrogenation of the β-ketoester precursor 10 furnishes 3 with only poor stereoinduction (14% ee) as a mixture with its (1R,2R)-diastereomer 12 [20]. The enzymatic variants are laborious. After reduction of 10 with sodium borohydride and acylation of the resulting four stereoisomers, treatment with lipases recovers 3 from the reaction mixture in 35–50% yield (45–50% conversion) with 86–95% ee [21]. The long reaction times before workup of 3–10 days and the low conversion rates are prohibitive for commercial applications.

Yeast technology furnishes 3 in a multi-kilogram scale after complete conversion in 70% yield and good stereoselectivity (>93% ee, >97% de, Scheme 4).

The advantages of the bioprocess result from the short reaction times and the high selectivity and yield. To our knowledge, bioreduction with baker's yeast currently is the best technology for producing 3. Needless to say, this process is working well commercially.

**Bistereogenic Asymmetric Bioreduction**

The impressive enantioselectivity of enzyme-mediated stereoselective reductions in living cells is finely represented by the two examples mentioned above which have in common that only one stereocentre is generated per reduction step. The bioreduction of acetonilacetone (4) to hexanediol (2) has to be regarded as a monostereogenic reduction, as the two keto groups are reduced subsequently. Moreover, they are not positioned vicinally, hence there is no influence of the keto-enol tautomerism on the outcome of the stereoinduction of the reduction reaction.

The bistereogenic enanti- and diastereoselective formation of α-substituted β-hydroxyesters has attracted much attention because of their widespread applicability in biologically active substance synthesis [22]. Although a variety of chemical methods has been developed for the enantioselective preparation of α-
Scheme 5: Stereoselectivity of the reduction of 10

\[
\begin{align*}
(R)-10 & \quad \xrightarrow{\text{enz}} \quad (3) + (12) \\
\text{10} & \quad \xrightarrow{\text{enz}} \quad \text{11} \\
(S)-10 & \quad \xrightarrow{\text{enz}} \quad (13) + (14)
\end{align*}
\]

Scheme 6: No enol-reduction of the enolester 15

\[
\begin{align*}
\text{10} & \quad \xrightarrow{\text{enz}} \quad \text{15} \\
& \quad \xrightarrow{\text{enz}} \quad \text{16} \\
& \quad \xrightarrow{\text{enz}} \quad \text{not observed}
\end{align*}
\]

However, both 13 and 14 are observed in the crude product mixture. Obviously, competing pathways or less stereoselective enzymes exist that also reduce the (S)-configured ketoester (S)-10. Remember (R)-10, 11 and (S)-10 are members of a fast-establishing equilibrium.

The answer is given by the observation that 3 is reoxidized by living yeast cells, whereas 3 is stable under oxidizing conditions in the absence of microbes. Moreover, if pure 3 is added to baker's yeast, not only the reoxidized ketone 10 is observed, but also significant amounts of 13 and 14 are formed. This strongly indicates that the product 3 itself induces a competing enzymatic pathway giving rise to the reduction of (S)-10 to the stereoisomers 13 and 14.

For these reasons, possibilities for the selective inhibition of the reduction of (S)-10 were investigated. An inhibition of the reoxidation reaction makes no sense, as the presence of unreduced ketone 10 would result in the formation of 13 and 14 in any case, once this pathway is open. A comparison of the reduction of 10 with and without the presence of an inhibitor is given in Table 2.

As shown in Table 2, there is no change in the relative amounts of 14, whereas the formation of 13 from (S)-10 could be reduced markedly by ca. 75%.

Selectivities

Besides the rather complex matter of stereoinduction in the reduction of 10, there are side reactions which also have an influence on the total yield of 3. The most important side reaction is ester hydrolysis. The saponification of 3 and 10 leads to the respective acids 17 and 18 of which the ß-ketoacid 18 decarboxylates spontaneously to give cyclo-
hexanone 19 (Scheme 7). The choice of the proper reaction conditions allows these by-products to be suppressed to an insignificant extent.

Conclusions

The three examples have shown that biotechnological processes are indeed viable in commercial industrial chemistry. Example 3 has shown that microbial reactions can be understood to a great extent and thus easily steered and controlled. With its consumption of regenerative raw material only and a mostly complete regeneration of volatile organic compounds, such as solvents for extractions, microbial synthesis is an important contribution to Green Chemistry and production-integrated environmental protection. Yeast is a versatile reagent for asymmetric synthesis and therefore microbial technologies can be both alternatives and complements to catalytic processes. With respect to all this, biotechnological reactions are of steadily growing acceptance and their meaning in industrial Green Chemistry will increase.

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Scheme 7: By-product formation during enantioselective microbial reduction of 10