BAKER'S YEAST CATALYZED ASYMMETRIC REDUCTION OF ETHYL 3-OXO HEXANOATE IN GLYCEROL CONTAINING SYSTEMS

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ABSTRACT: Organic synthesis is usually performed in solution to dissolve both reactants and catalysts and
to deliver heat. Glycerol is a non-toxic, biodegradable, and recyclable liquid manufactured from renewable
sources. It has a high potential to serve as alternative green solvent for organic reactions. Several catalytic and
non-catalytic reactions were successfully performed in glycerol. High products yields and selectivities were
achieved. Present work describes the enantioselective reduction of keto esters by baker’s yeast in glycerol:
water mixture in different proportions. And results show that a mixture of glycerol and water combined the
advantageous of each individual solvent and resulted in high catalytic performance and efficient product
extraction yield.

Keywords: biodegradable, baker's yeast, glycerol, enantioselective reduction, catalytic performance.

INTRODUCTION

Solvents are used daily in numerous industrial processes as reaction medium, in separation procedures, and as
diluters. As reaction medium, solvents are employed to bring reactants and/or catalysts together and to deliver
heat and momentum. In addition, the solvent may also affect activity and selectivity. Selection of solvent is
made on the basis of its chemical, physical, and biological nature, also plays a key role from environmental,
economic, safety, handling, and products isolation point of views. Water is the first solvent of choice regarding
the aforementioned considerations, yet the negligible solubility of many organic and organo-metallic
compounds in water limits its applications.

The asymmetric reduction of ketoesters to their corresponding chiral hydroxy esters was extensively studied
with both free and immobilized Baker's yeast cells (FBY and IBY correspondingly) (Scheme-1). Though water
is the first solvent of choice for biocatalysis, the low solubility of many organic molecules in water, the
existence of undesired side reactions such as hydrolysis, and difficult product separation procedure, limit its
applications. Replacing water with organic solvent may overcome these problems and also assist with product
recovery. Different organic solvents such as hexane, toluene, ethyl acetate, petroleum ether, and liquefied
petroleum gas as well as ionic liquids, and fluorous media, which offer recyclable and more environmental
friendly organic media, were tested for this purpose. However, not only that using organic solvent has negative
environmental impact, it also affects the yeast cell viability. In addition, glucose which is usually added as
carbon and hydrogen source and as electron donor in the regeneration of the co-factor in baker's yeast
reductions has negligible solubility in organic solvents and thus results in low reaction rates.
Scheme-1  Depicting biological pathway for reduction of carbonyl group by NADH

Recent researches show that the promising physical and chemical properties of glycerol make it an ideal reaction medium for various catalytic and non-catalytic organic syntheses\(^2-3\). Specifically, it has a high boiling point and negligible vapor pressure, it is compatible with most organic and inorganic compounds, and it does not require special handling or storage. Similar to other polar organic solvents such as DMSO and DMF, glycerol facilitates the dissolution of inorganic salts, acids, and bases, and of enzymes and transition metal complexes. Furthermore, it also dissolves organic compounds that are poorly miscible in water and it is considered non-hazardous. Hydrophobic solvents such as ethers and hydrocarbons, which are immiscible in glycerol, enable the products to be removed by simple extraction. Additionally, the high boiling point of glycerol makes distillation of the products also a feasible separation technique. Finally, glycerol has successfully been employed as a versatile and alternative green solvent in a variety of organic reactions and synthesis methodologies, in all of which high product conversions and selectivities were achieved.

In present work utility of glycerol for baker’s yeast catalyzed reduction of keto ester, ethyl 3-oxo hexanoate was studied. Glycerol, which is a polar, non-toxic, biodegradable and recyclable liquid manufactured from renewable sources, was used as alternative green organic solvent for several organic reactions. The high polarity of glycerol allowed dissolving glucose, sucrose or isopropanol as energy source. Moreover it allowed easy separation of the product by extraction with glycerol immiscible solvents. It was found that employing both FBY and IBY resulted in high enantioselectivity (>95%) and reasonable activity for various keto esters and ketones. Also immobilization of the yeast cells keeps some water around them and thus increases their performance.
RESULTS AND DISCUSSION
The investigation initiated by preceding the enantioselective reduction of ethyl 3-oxo hexanoate to ethyl-3-hydroxyhexanoate with both FBY and IBY in pure water and mixture of glycerol: water in different proportions under similar conditions (fig.1). The extraction yields of the product ethyl 3-hydroxyhexanoate obtained by reduction with FBY were tested with dichloromethane and diethyl ether. As illustrated in fig.1, it was found that higher extractions yields were obtained in mixtures that contained 35-65 wt. % of glycerol in water. Maximum yield is obtained for 50-55 wt% of glycerol. In these mixtures, the extractions yields were increased by ~35% when compared to each individual solvent. This maximum can be explained by formation of new interactions between glycerol and water, which decreased the solubility of product in the mixture.

![Ethyl 3-hydroxyhexanoate extractions yields in water and glycerol mixtures](image)

The enantioselectivity of the product, which is the most important value in asymmetric synthesis, was very high in all the reactions. Results clearly show that yields were high when reaction was performed in glycerol: water (50:50) mixture. Furthermore yields with IBY are much higher than with FBY. It can be attributed to the high amount of water, which was left inside the IBY beads during the preparation procedure as a minimum amount of water (hydration shell) is required around the cells for correct functioning of cell processes and enzyme activities. Furthermore, as illustrated in table- 1, addition of isopropanol to the reaction mixture in glycerol slightly increased the conversion of ethyl 3-oxo-hexanoate. It might be attributed to enhanced regeneration of the co-factor in baker's yeast in the presence of isopropanol that uses as electron donor.

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**Fig-1 Ethyl 3-hydroxyhexanoate extractions yields in water and glycerol mixtures**

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Table-1 Comparison of the asymmetric reduction of ethyl 3-oxo hexanoate in water and glycerol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Energy Source</th>
<th>Water Yield (%)</th>
<th>ee (%)</th>
<th>Glycerol-water (50:50) Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FBY</td>
<td>No</td>
<td>86</td>
<td>&gt;95</td>
<td>88</td>
<td>&gt;98</td>
</tr>
<tr>
<td>2</td>
<td>IBY</td>
<td>No</td>
<td>90</td>
<td>&gt;95</td>
<td>94</td>
<td>&gt;98</td>
</tr>
<tr>
<td>3</td>
<td>FBY</td>
<td>Isopropanol</td>
<td>92</td>
<td>&gt;95</td>
<td>96</td>
<td>&gt;98</td>
</tr>
<tr>
<td>4</td>
<td>IBY</td>
<td>Isopropanol</td>
<td>95</td>
<td>&gt;95</td>
<td>99</td>
<td>&gt;98</td>
</tr>
</tbody>
</table>

Finally, employing glycerol as the solvent also allowed easy separation of the product by simple extraction with a glycerol immiscible solvent such as diethyl ether or dichloromethane (table2). The effect of the extracting solvent type and extraction procedure on extractions yields of ethyl 3-hydroxyhexanoate was tested after the reaction reached full conversion. Several representative glycerol and water immiscible solvents were examined as illustrated in table 2. As illustrated in fig.1 and results shown in table-2, employing diethyl ether as extracting solvent resulted in slightly higher extraction yields for all the mixtures. In general it can be seen that for each solvent the extraction yield with glycerol were slightly higher than the extraction yield with water. In addition, as expected, increasing the extractions steps proportionally increased the product extraction yield (table 2, entries 3-5). Moreover, in contrast to extraction from water, glycerol did not form emulsion with the extracting solvents and hence the separation was simpler.

Table-2 Comparison of ethyl 3-hydroxyhexanoate recovery from Water and Glycerol-water mixture.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Extracting Solvent</th>
<th>Steps</th>
<th>Extracting Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>1</td>
<td>n-Hexane</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>Dichloromethane</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>Diethyl ether</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>Diethyl ether</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>5</td>
<td>Diethyl ether</td>
<td>3</td>
<td>92</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS
All the chemicals were used of AR grade and triply distilled water was used for the making of solution and Baker’s Yeast is purchased from the glossary shop.

Experimental
Immobilization of BY in polyacrylamide gel: -The gel was prepared using the following solutions.
Solution A: - Acrylamide (10 g) and N, N’-methylene bisacrylamide (2.5 g) in DD (100 ml), Solution B: - Tris (5.98 g), TEMED (0.46 ml) and 1N HCl (48 ml) solution to 100ml, Solution C: -APS (560 mg) in DDW (100 ml), Solution D: - Isopropanol (25 ml).
Where- TRIS= Trihydrixy Methyl Amino Methane, TEMED= N, N, N’, N”-tetramethyl Ethylenediamine, APS= Ammonium Persulphate, DDW= doubly distilled water.
Then Solutions were mixed in following way- Sol. A (10 ml) + sol.B (5 ml) + BY (2g) + sol.C (5 ml).
And then solution D was added and then deaerated for 30min
Asymmetric reduction
The asymmetric reduction of compound with FBY and IBY in glycerol was performed as follows: 10 g of FBY or 50 g of IBY were added to a mixture of 50 mL of solvent (glycerol-water mixture) in a one liter round bottom flask, equipped with a magnetic stirrer (Remi-2MLH make) and stirred for 30 min. Then 25 ml of isopropanol was added and then again stirred for extra 10 min before 1g of compound was added. Then reaction mixture was shaken at for 48-96 h. The suspension changed its colour during the course of reaction. After completion of the reaction, the product was filtered using celite (HIMEDIA grade), the filtrate was saturated with NaCl and the product was extracted with diethyl ether in three steps (3X40 ml). Ether extracts were combined and dried over sodium sulphate. After evaporation, the product was isolated, purified and characterized by combined application of chromatographic techniques and spectroscopy.
Characterization of the Product

The purity of products was checked by single spot obtained by (Thin layer chromatography) TCL and then characterization of products was carried on the basis of IR & NMR spectral analysis. NMR spectra were recorded in CDCl$_3$ solution on Joel (Japan) 300 MHz spectrophotometer and IR spectra were recorded by using Nicolet (USA) FTIR Spectrophotometer. Samples were sent to CDRI for mass spectral analysis. Optically activity of products was measured by using a JASCO P-2000 polarimeter and enantiomeric excess was calculated. Spectroscopic results are shown in table-3.

<table>
<thead>
<tr>
<th>Table-3 Spectroscopic data for microbial reduction of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Ethyl 3-hydroxyhexanoate</td>
</tr>
</tbody>
</table>

FBY=Free Baker’s Yeast, NADH=Nicotinamide adenine dinucleotide hydride, IBY=Immobilized Baker’s Yeast, DMSO=Dimethyl sulfoxide, DMF=Dimethyl formamide, ee =Enantimeric excess, TRIS= Trihydroxy Methyl Amino Methane, TEMED = N, N, N’, N”-tetramethyl Ethylenediamine, APS= Ammonium Persulphate, DDW= doubly distilled water

Conclusions

Based on the above results, it is clear that while water is the natural and most suitable solvent for biocatalysts from the viability and activity points of view, glycerol is advantageous in substrate solubility and product separation procedure. Hence performing the asymmetric reduction in a mixture of water and glycerol might combine the advantageous of both individual solvents.

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REFERENCES