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Bioreduction of a carbon–nitrogen double bond using immobilized baker's yeast—a first report

S.S. Chimni* and R.J. Singh

Immobilized baker's yeast entrapped in calcium alginate beads efficiently reduces *N*-benzylidinemethylamine to *N*-methylbenzylamine in hexane at 37 °C and tetrahydrofuran (THF) at 30 °C in the presence of 18-crown-6, while in the presence of water as cosolvent and glucose as an additive *N*-benzylidinemethylamine undergoes decomposition. Benzaldoxime in a hexane–water (1:9) solvent system containing glucose as an additive is reduced to *N*-benzylhydroxylamine. On using an ethanol–water (1:1) solvent system, benzaldoxime is converted to benzyl alcohol and in hexane, benzene, THF, hexane–water (1:1) or acetonitrile–water (1:1) solvent systems, or using dried baker's yeast in different solvent systems, transformation of benzaldoxime does not occur.

Key words: Baker's yeast, 18-crown-6, imines, immobilization, oximes.

Baker's yeast has emerged as a useful reagent for the reduction of unnatural organic ketones, β-ketoesters, α,β-unsaturated systems and polarized carbon–carbon double bonds (Csuk & Glanzer 1991; Servi 1990). Although the literature contains many examples of these reductions, other potentially reducible polarized double bonds, for example C=N, have been relatively little studied. There is no report on the reduction of imines and only isolated reports are available on the transformation of oximes to amines rather than hydroxylamine (Gibbs & Barnes 1990) and hydrolysis to starting ketones/aldehydes (Kamal *et al*. 1991). In view of the versatility of baker's yeast in the reduction of organic functional groups and the well known role of L-glutamate dehydrogenase in the reduction of the C=N bond in both catabolic and anabolic transformations of amino acids (Lehninger 1982), we planned to investigate the potential of baker's yeast in the reduction of the C=N bond. In this communication we report our results on the bioreduction of *N*-benzylidinemethylamine and benzaldoxime in aqueous, aqueous–organic and organic media. This constitutes the first examples of bioreduction to amines and hydroxylamine derivatives.

S.S. Chimni and R.J. Singh are with the Department of Chemistry, Guru Nanak Dev University, Amritsar 143 005 India; fax: 183 258820. *Corresponding author.

Materials and methods

N-Benzylidinemethylamine, $C_6H_5CH{=}N\text{-}CH_3$ (Moffett, 1963), and benzaldoxime, $C_6H_5CH{=}N\text{-}OH$ (Furniss *et al*. 1978), were prepared by the reported procedures from commercially available benzaldehyde, methylamine and hydroxylamine hydrochloride. Authentic samples of the reduced products were also prepared by reported procedures (Feuer *et al*. 1965). ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on Brucker AC 200 MHz instrument. The samples were dissolved in $CDCl_3$. (Aldrich Chemical Co.) containing tetramethylsilane as internal standard. Mass spectra were recorded on Shimadzu QP 2000 Mass Spectrometer. The progress of the reaction was monitored using silica gel G- or HF 254-coated glass chromatographic plates. Column chromatography was performed on a column (length 300 mm., diameter 15 mm) packed with 60–120 mesh silica gel (Acme India Ltd) using mixture of hexane, ethyl acetate and methanol as eluents.

Preparation of Immobilized Baker's Yeast (ImBY) Entrapped in Calcium Alginate Beads

Sodium alginate (5 g) was dissolved in distilled water (200 ml) by additions in small portions. The resulting solution was added to a suspension of baker's yeast (15 g) in distilled water (75 ml). The mixture was stirred until it became homogeneous and was rapidly added to a 2% aqueous solution of $CaCl_2$ with a syringe mounted with a needle. The immobilized baker's yeast was obtained as beads which were filtered and used for further reactions (Bucke 1987).

General Procedures for Bioreduction of N-*Benzylidinemethylamine (***1***) and Benzaldoxime (***3***) with Immobilized Baker's Yeast (ImBY)*

Water. A mixture of dried baker's yeast (20 g) and glucose (15 g) in distilled water (200 ml) was shaken at 32 °C for 30 min. To this fermenting mixture was added substrate, *N*-benzylidinemethylamine (**1**) and benzaldoxime (**3**) (8 mmol) dissolved in ethanol (5 ml) and the mixture was shaken for 5–7 days. The mixture was then filtered through celite and the filtrate extracted with ethyl acetate. Work-up of the extract gave a crude product, which was purified by column chromatography and then analysed by ^{1}H NMR and mass spectrometry.

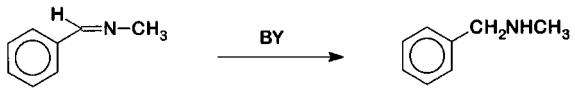
Organic-Water Solvent System. A mixture of ImBY (prepared from 15 g of dried BY) or dried baker's yeast (20 g), the additive and the substrate **1** or **3** (8 mmol) in the respective organic–water solvent system was shaken at 30 °C or 37 °C for 3–7 days. The progress of the reaction was monitored by thin-layer chromatography (TLC). After the completion of the reaction, or if it was not complete then after 7 days, the beads were filtered and washed with the solvent system. The combined filtrate and washings were extracted with ethyl acetate and dried over anhydrous sodium sulphate. The solvent was distilled to obtain the crude reaction mixture, which was purified by column chromatography and analysed by ^{1}H NMR and mass spectrometry.

Organic Medium. A solution of compounds **1** or **3** (8 mmol) in an organic solvent was shaken at 30 °C or 37 °C with ImBY (prepared from dried baker's yeast, 15 g) or dried baker's yeast (20 g) along with an additive for 5–7 days. The reaction mixture was filtered and ImBY beads washed with the solvent, from which the crude product was obtained by distillation. The crude mixture was purified by column chromatography and products analysed by ^{1}H NMR and mass spectrometry.

Results and Discussion

Bioreduction of N-*benzylidinemethylamine* (***1***)

N-Benzylidinemethylamine was chosen as a representative compound for performing yeast-mediated reduction of imines. A solution of 8 mmol of compound **1** in 5 ml of ethanol was added to the fermenting mixture consisting of 20 g of dried baker's yeast and 15 g of glucose in 200 ml distilled water and the mixture was shaken on an orbital shaker. After 7 days, it was filtered, with difficulty, and the filtrate was extracted with ethyl acetate. The distillation of the solvent gave a liquid residue, which on comparison (TLC) with authentic samples of compounds **1** and **2** and ^{1}H NMR spectra did not show the presence of either the substrate or the product. To overcome the difficulty in work-up and a possible hydrolysis of the substrate under hydrolytic conditions, for further reactions, the freshly prepared immobilized baker's yeast entrapped in calcium alginate beads of 2 mm diameter in a solvent system consisting of water/organic solvent was used.



(1) (2)

A solution of *N*-benzylidinemethylamine (1 g, 8 mmol) in different organic–water solvent systems (Table 1) and freshly prepared beads of immobilized baker's yeast prepared from 15 g of dried baker's yeast were shaken at 30 °C for 5–7 days along with an additive such as glucose, 18-crown-6 or acetic acid. Using the organic–water system and glucose neither the product nor the reactant could be detected in the reaction mixture. Because imines are sensitive to hydrolysis in aqueous systems, compound **1** probably undergoes decomposition under these conditions. So it was planned to perform the reduction of compound **1** in an organic solvent. On performing the reduction of compound **1** in tetrahydrofuran (THF), **1** could be recovered untransformed.

Additives are known to play an important role in controlling the baker's yeast-mediated transformations in organic solvents (Naoshima *et al*. 1992). The role of crown ethers in enhancing the rate of enzymatic reactions has been highlighted by Tsukube *et al*. (1994). In order to study the effect of crown ethers in controlling the bioreduction, the reaction was carried out using 18-crown-6 (18-C-6) as an additive. Shaking a suspension of ImBY in the solution of compound **1** and 18-crown-6 for 7 days at 30 °C in THF, followed by the usual work-up and column chromatography, provided the reduced product *N*-methylbenzylamine (**2**). The formation of compound **2**

Table 1. Immobilized baker's yeast-mediated reduction of *N*-benzylidenemethylamine (1) to *N*-benzylmethylamine (2).

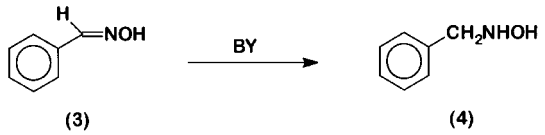
Entry	Solvent system	Additive	Temperature (°C)	Time (days)	Product
1	Ethanol–water (1:1) 400 ml	Glucose (5 g)	30	7	–
2	Acetonitrile–water (1:1) 400 ml	Glucose (5 g)	30	7	–
3	Hexane–water (1:1) 400 ml	Glucose (5 g)	30	7	–
4	Tetrahydrofuran (400 ml)	–	30	7	**1**
5	Tetrahydrofuran (400 ml)	18-C-6 (20 mg)	30	7	**2**
6	Hexane (400 ml)	18-C-6 (20 mg)	30	7	**1**
7	Hexane (400 ml)	18-C-6 (20 mg)	37	5	**2**
8	Hexane (400 ml)	Acetic acid	30	7	–

has been confirmed by comparison of the product with authentic sample (TLC) and its ^{1}H NMR and mass spectra. Its ^{1}H NMR spectrum shows two singlets at δ 2.5 and 3.6 along with aromatic signals at δ 7.3, which indicate the formation of amine **2**. In its mass spectrum, the presence of the parent ion peak at *m/z* 121 [M^+] and a base peak at *m/z* 91 corresponding to the tropolium ion, further confirm the formation of *N*-methylbenzylamine (**2**). On shaking the solution of compound **1** in hexane containing immobilized baker's yeast and 18-crown-6 there was no formation of product **2** at 30 °C, but on increasing the temperature to 37 °C, **2** was formed in 5 days.

Thus, for the immobilized baker's yeast-mediated reduction of *N*-benzylidinemethylamine (**1**) to *N*-methylbenzylamine (**2**), the optimum conditions are aprotic organic solvents such as hexane (37 °C) or THF (30 °C) in the presence of 18-crown-6 as additive. This constitutes the first example of baker's yeast-mediated bioreduction of the carbon–nitrogen double bond.

Bioreduction of Benzaldoxime (**3**):

The reports on the biotransformation of oximes using baker's yeast have provided conflicting results. Dried baker's yeast in sodium acetate buffer transforms oxime ethers and *O*-acetyl derivatives to amines, the N—O bond is cleaved in this process and the expected hydroxylamine derivatives are not formed (Gibbs & Barnes 1990). Ultrasonically pretreated dried baker's yeast in phosphate buffer causes hydrolysis of oxime to the corresponding aldehydes or ketones (Kamal *et al*. 1991). We envisaged that the conditions used for bioreduction of imines may be useful for reduction of oximes to *N*-alkylhydroxylamines.



(3) (4)

During bioreduction of benzaldoxime in hexane containing freshly prepared beads of immobilized baker's yeast (prepared from 15 g of dried baker's yeast) and 18-crown-6, after 7 days the reaction did not show any formation of *N*-benzylhydroxylamine and the oxime was obtained untransformed. Other solvent systems including organic (benzene, THF) and organic–water (hexane–H_2O, CH_3CN–H_2O, ethanol–H_2O) with additives, were then selected for the reduction of compound **3** (Table 2). The hexane–H_2O (1:9) system containing glucose as an additive yielded *N*-benzylhydroxylamine (**4**) after 3 days whereas the hexane–H_2O (1:1) mixture (Table 2, entry 4) yielded the untransformed oxime. The formation of compound **4** was evident from its comparison with the authentic sample (TLC), ^{1}H NMR and mass spectra. The ^{1}H NMR of the product shows a 2H singlet at δ 3.5 and a 5H singlet at δ 7.5 and confirms the formation of compound **4**. The parent ion peak at *m/z* 123 [M^+] in the mass spectrum further supports the formation of compound **4**.

The bioreduction of compound **3** in an ethanol–H_2O mixture resulted in the formation of benzyl alcohol. Its formation has been confirmed by its comparison with an authentic sample (TLC) and ^{1}H NMR which shows a 2H singlet at δ 4.5 and a 5H singlet at δ 7.5. Further attempts were made to reduce the oxime by using dried baker's yeast under fermenting conditions and in an organic–water solvent system (Table 3). In fermenting conditions

Table 2. Immobilized baker's yeast-mediated reduction of benzaldoxime (3) to *N*-benzylhydroxylamine (4).

Entry	Medium	Additive	Temperature (°C)	Time (days)	Results
1	Hexane (400 ml)	–	30	7	**3**
2	Benzene (400 ml)	–	30	7	**3**
3	Tetrahydrofuran (400 ml)	18-C-6	30	7	**3**
4	Hexane–water (1:1) 250 ml	Glucose (2.5 g)	30	7	**3**
5	Hexane–water (1:9) 500 ml	Glucose (5 g)	30	3	**4**
6	Acetonitrile–water (1:1) 400 ml	Glucose (5 g)	30	7	**3**
7	Ethanol–water (1:1) 400 ml	Glucose (5 g)	30	7	Benzyl alcohol
8	Ethanol–water (1:1) 400 ml	Acetic acid (pH 5)	30	7	Benzyl alcohol

Table 3. Dried baker's yeast-mediated [a] reduction of benzaldoxime (3).

Entry	Medium	Additive	Temperature (°C)	Time (days)	Results
1	Water (200 ml)	Glucose (15 g)	32	5	Benzyl alcohol
2	Ethanol–water (1:1) 400 ml	Glucose (5 g)	30	7	–
3	Acetonitrile–water (1:1) 400 ml	Glucose (5 g)	30	7	–
4	Tetrahydrofuran (400 ml)	18-C-6 (20 mg)	30	7	**3**
5	Acetonitrile (300 ml)	Acetic acid (cat.)	30	7	**3**

[a] 20 g dried Baker's yeast was used for each reaction.

benzyl alcohol was formed and in organic–water solvent system nothing could be isolated. Thus, shaking a solution of benzaldoxime in hexane–H_2O (1:9) containing glucose and ImBY at 37 °C was found to be the optimum condition for bioreduction of benzaldoxime.

Benzylidinemethylamine (**1**) which is soluble in hexane but undergoes hydrolysis in ethanol or water is best reduced by using hexane or THF as solvents. In contrast oxime **3**, which is insoluble in hexane, benzene or THF is not reduced in these solvents but on using water as cosolvent, is reduced efficiently to *N*-benzylhydroxylamine. Hence, solubility and stability of the substrate in the solvent system is one of the essential requirements for performing these reductions.

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References

Bucke, C. 1987, Cell immobilisation in calcium alginate. *Methods in Enzymology* **135**, 175–89.

Csuk, R. & Glanzer, B.I. 1991 Baker's yeast mediated transformations in organic chemistry. *Chemical Reviews* **91**, 49–97.

Feuer, H., Vincent Jr, B.F. & Bartlett, R.S. 1965 The reduction of oximes with diborane. A new synthesis of *N*-monosubstituted hydroxylamines. *Journal of Organic Chemistry* **30**, 2877–2880.

Furniss, B.S., Hannaford, A.J., Rogers, V., Smith, P.W.G. & Tatchell, A.R. 1978 In *Vogel's Textbook of Practical Organic Chemistry Including Qualitative Organic Analysis*, 4th edn, pp 810, London: ELBS.

Gibbs, D.E. & Barnes, D. 1990 Asymmetric synthesis of amines by action of baker's yeast on oximes. *Tetrahedron Letters* **31**, 5555–5558.

Kamal, A., Rao, M.V. & Meshram, H.M. 1991 Enzymatic deoximation of oximes by ultrasonically stimulated baker's yeast. *Journal of Chemical Society Perkin Transactions* **1**, 2056–2057.

Lehninger, A.I. 1982 *Principles of Biochemistry*. Delhi: CBS Publishers & Distributors Pvt. Ltd.

Moffett, R.B. 1963 N-Methyl-1,2-diphenylethylamine and hydrochloride. In *Organic Synthesis Collective* Vol. 4, ed. Rabjohn N., pp. 605–607. New York: John Wiley & Sons Inc.

Naoshima, Y., Malda, J. & Munakata, Y. 1992 Control of the enantioselectivity of bioreduction with immobilized baker's yeast in hexane solvent system. *Journal of Chemical Society Perkin Transactions* **1**, 659–660.

Servi S. 1990 Baker's yeast as a reagent in organic synthesis. *Synthesis* 1–25.

Tsukube, H., Betchaku, A., Hiyama, Y. & Itoh, T. 1994 Lipase catalysed hydrolysis of crowned ester substrate:metal cation-enhanced reactivity and enantioselectivity of 12-crown-4-esters. *Journal of Organic Chemistry* **59**, 7014–7018.

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