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Concerning the Baker's Yeast (*Saccharomyces cerevisiae*) Mediated Reduction of Nitroarenes and Other N-O Containing Functional Groups

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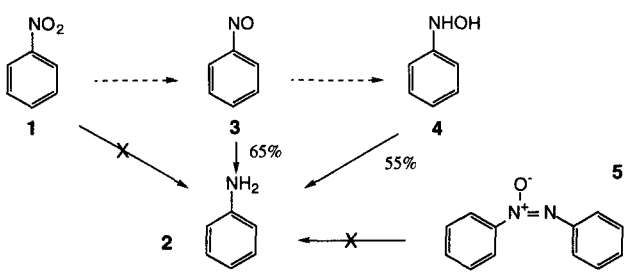
Abstract: Nitro- and nitrosoarenes can be reduced using baker's yeast (*Saccharomyces cerevisiae*) under two distinct sets of conditions, one of which is in fact a well established non-enzymic process. In order to clarify reports in the literature a comparison of the two methods has been made. © 1997 Elsevier Science Ltd.

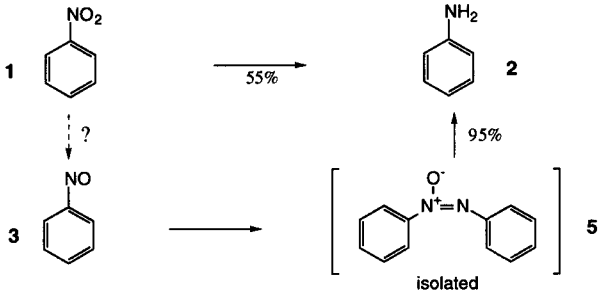
Recently there have been a number of reports concerning the use of baker's yeast (*Saccharomyces cerevisiae*) for the reductive cleavage of N-O bonds in a variety of functional groups including nitro arenes[1], nitroalkenes[2], nitrosoarenes[3], isoxazoles[4] and *N*-oxides[5]. In general the reactions proceed under mild conditions and may present synthetic advantages in terms of chemo- and regioselectivity. Typically yeast catalysed reactions are carried out at neutral pH in aqueous media with a substrate concentration of 1-2 mg ml^{-1}. However, inspection of the literature relating to yeast catalysed N-O reductions revealed that two distinct sets of reaction conditions could be employed for this biotransformation. Whereas most of the papers [1a-d, 2b, 4, 5a] describe the use of *S. cerevisiae* at pH 5.5-6.0 and 30 °C with fermenting or non-fermenting yeast (thereafter called type I conditions), the group of Baik *et al.*,[1e-f, 2a, 3, 5b] report quite different reaction conditions, namely reaction temperatures of 70-80 °C and even reflux (*sic*)[5b], high pH (> 12) and the inclusion of methanol/ethanol in the reaction medium (type II conditions). Even with respect to the reduction of nitroarenes (*vide infra*), the two different reaction conditions result in different selectivity, *e.g.* under type I conditions electron-withdrawing groups are required for successful reduction [1a,b] whereas with type II conditions electron-donating groups can be tolerated.[1e] This discrepancy suggested to us a difference in reaction mechanism, especially in view of the fact that the yeast is unlikely to be stable at high pH and temperature. In this letter we report on our investigations into the likely mechanisms of these two reductions.



Scheme 1: Reduction of nitrobenzene and derivatives using *S. cerevisiae* under type I conditions.
typical conditions: *S. cerevisiae* (20g), substrate (100 mg), water (100 ml), 30 °C, pH 5.5-6.0.

An indication of the difference between the two sets of conditions can be gained by comparing the reduction of nitrobenzene **1** (**Schemes 1** and **2**). Under type I conditions, no reduction of nitrobenzene

can be detected whereas with type II conditions a 55% yield of aniline **2** is obtained. Use of nitrosobenzene **3** with type I conditions gives a clean conversion to aniline (65%) whereas under type II conditions, modified by exclusion of the NaOH, the azoxybenzene **5** is detected as an intermediate *en route* to aniline. By comparison, azoxybenzene is not reduced under type I conditions. We have also shown that phenylhydroxylamine **4**[6] can be reduced under type I conditions to give aniline in 55% yield leading us to propose the sequence of events shown in **Scheme 1**.

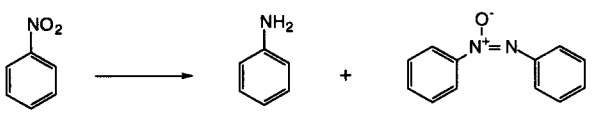


Scheme 2: Reduction of nitrobenzene and derivatives using *S. cerevisiae* under type II conditions.
typical conditions: *S. cerevisiae* (30g), substrate (500 mg), NaOH (4g) (except for nitroso reduction), water (900 ml), methanol (40 ml), 70-80 °C, pH >12.

In order to gain further insight into the difference between the two sets of conditions we subjected 2,4-dinitroanisole to the reduction. Using our optimised protocol for carrying out these yeast catalysed reductions[7] we obtained a 5.3:1 ratio of 2-amino-4-nitroanisole : 2-nitro-4-aminoanisole in a combined yield of 95%. However, under type II conditions the reaction was found to be less clean and resulted only in isolation of 2-amino-4-nitroanisole in a yield of 20%.

In addition to the evidence provided above, the following points should be noted. Firstly, under type II conditions, it is highly unlikely that the yeast cells remain active during the reaction. Indeed, our experience has been that the cells appear to coagulate very rapidly presumably followed by cell death and subsequent inactivation of the enzyme activity under the high pH and high temperature of the medium. Secondly, it is well known that nitroarenes can be reduced to the corresponding anilines and azoxybenzenes under strongly basic conditions in solutions that contain simple alcohols and/or glucose or fructose.[8] It thus occurred to us that perhaps the *S. cerevisiae* type II conditions were in fact simply a variation of these classical conditions and that the yeast was merely acting as an alternative source of carbohydrate.

In order to test this notion we carried out a series of comparative experiments involving the reduction of nitrobenzene under a variety of conditions as shown in **Scheme 3**. The principal observation from these experiments is that the effect of adding glucose to the reaction at the appropriate concentration is essentially the same as that of adding *S. cerevisiae*, leading to the conclusion that the function of the yeast is to act as a source of carbohydrate.

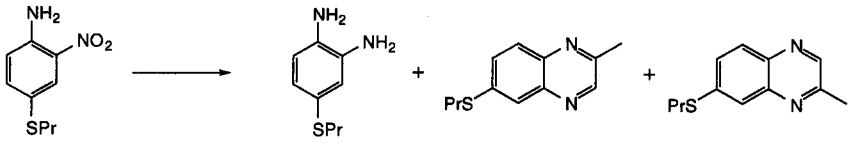


conditions	yield/%	
no NaOH, glucose	0	0
NaOH (5g), glucose (1g)	11	60
NaOH (5g), glucose (5g)	30	0
NaOH (5g), *S. cerevisiae*	30	0

Scheme 3: Reduction of nitrobenzene.
conditions: nitrobenzene (1g), water (80 ml), MeOH (40 ml).

We considered the possibility that the use of *S. cerevisiae* under type II conditions may offer some advantage over simple addition of glucose in that the carbohydrate may be provided in a slow release form

as the yeast cells degrade. Some support for this proposal was obtained from the reduction of the nitroarene **6** (**Scheme 4**). The use of *S. cerevisiae* resulted in a cleaner reaction (59% of the aniline **7**) compared to the use of glucose/fructose which gave a lower yield of the aniline and a 30% yield of the quinoxalines **8** and **9**. It is noteworthy that nitroaniline **6** was totally inactive under type I conditions providing further evidence for the difference between type I and II conditions.

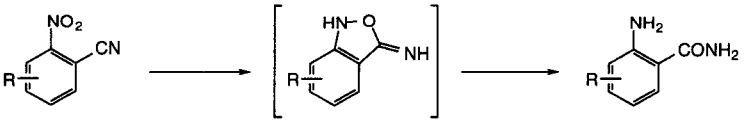


6 **7** **8** **9**

conditions	yield/%		
S. cerevisiae (30g)	59	0	
glucose/fructose (4g)	trace	30	

Scheme 4: Reduction of nitroaniline **6**.
conditions: substrate (1g), water (80 ml), MeOH (40 ml), NaOH (5g).

The presence of arylhydroxylamines as intermediates in the reduction pathway under type I conditions has been demonstrated above (**Scheme 1**) and inferred from previous experiments[1a] in which it was found to be possible to reduce 1,2-nitrocyanoarenes to the corresponding 1,2-aminobenzamides, presumably *via* the corresponding isoxazoline intermediates (**Scheme 5**). We have extended this approach by investigating the reduction of some dicyanonitroarenes and have found that in all cases the nitrile group *ortho*- to the cyano group undergoes selective transformation to the benzamide resulting in a means for selectively manipulating one nitrile group in the presence of another.



R	time	yield/%
H		79
3-CN		75
4-CN		--
5-CN		84
4-Cl		69
5-CF_3		84

Scheme 5: Conversion of 1,2-nitrocyanoarenes to 1,2-aminobenzamides.

In conclusion it seems clear that the baker's yeast mediated reduction of nitroarenes and related N-O containing substrates is best carried out under conditions that maintain the integrity of the yeast thereby exploiting the inherent enzymic catalytic activity. In our hands, the reactions using type I conditions are simpler and cleaner and result in higher yields. Regarding the type II conditions it seems that there is no significant advantage in using baker's yeast over glucose/NaOH/MeOH for simple nitro reductions. Moreover, any claims that there are advantages in terms of chemoselectivity (*i.e.* reduction of nitro groups in the presence of ketones[5b]) are almost certainly due to the destruction of all enzyme activity under the reaction conditions used leading to non-enzymic processes only. It is noteworthy that previous claims of baker's yeast mediated reactions have subsequently been revised by others in the light of more carefully executed experiments with the appropriate controls.[9]

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5. a) Takeshita, M.; Yoshida, S. *Heterocycles*, 1990, **30**, 871; b) Baik, W.; Kim, D.I.; Koo, S.; Rhee, Shin, S.H.; Kim, B.H. *Tetrahedron Lett.*, 1997, **38**, 845.
6. Phenylhydroxylamine is relatively unstable but can be prepared and conveniently stored as its *N,O-bis*-acetyl derivative; *Org. Syn.*, 1989, **67**, 187.
7. *Typical conditions for the baker's yeast reduction of nitroarenes*: The baker's yeast purchased from Sigma (Sigma type II) was initially purified using the following procedure (*N.B.* this purification of commercially available baker's yeast has been found to result in cleaner product isolation). To a solution of acetone (1l) at -20 °C was added baker's yeast (200-300g) and the suspension stirred gently for 20 minutes after which the acetone was removed by decanting and the procedure repeated with a further quantity of acetone (1l). After the second washing the yeast was collected and dried. Acetone-washed baker's yeast (10g) was suspended in tap water (40 ml) and incubated at ~32 °C for 1h, after which the substrate (100 mg) dissolved in DMSO or hot ethanol (~5 ml) was added. The reaction was shaken at 32 °C, in an orbital shaker, and the conversion monitored by t.l.c. Upon completion of the reaction, the aqueous medium was saturated with NaCl and the pH adjusted to 8. The entire mixture was then continuously extracted overnight with chloroform after which the chloroform layer was washed, dried and evaporated to yield the crude product. Standard chromatographic procedures lead to the isolation of the aniline products.
8. Vogel's *Textbook of Practical Organic Chemistry*, 4th Ed., p. 724.; Prato, M.; Quintily, U.; Scaplo, L.; Scorrano, G. *Bull. Chim. Soc. France*, 1987, N1, 99.
9. For a discussion of the purported baker's yeast catalysed 1,3-dipolar cycloaddition reactions of nitrile oxides see Easton, C.J.; Hughes, M.M.; Tiekink, E.R.T.; Savage, G.P.; Simpson, G.W. *Tetrahedron Lett.*, 1995, **36**, 629.

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